REVIEW ARTICLE

ACUTE INFLAMMATION

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A Review

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INFLAMMATION has been a favored topic for research in recent years. It has graduated to a three-volume treatise, duly bound in red;[1] symposia and monographs are plentiful;[2-9] there is an Inflammation Club, an Inflammation Bulletin;[10] and a new (red) Inflammation journal has just appeared.[11] There is even a book on the future trends in inflammation.[12]

To those who are not specialized, this avalanche of literature has become difficult to analyze; the present review was therefore conceived as a short and critical guide to that part of the field generally known as *acute inflammation*.

History—The Saga of the Fifth Sign

A history of inflammation starting from the earliest times can be found in a recent book, where it is interwoven with the history of the wound.[13] In summary, we can say that the idea of comparing a red, hot, swollen skin lesion to something ablaze may be as old as medicine. In the cuneiform writings of Mesopotamia, several medical terms can be translated as *inflamed* or *inflammation; ummu*, for instance, means "the hot thing" and is used in a context that suggests either local or general heat (inflammation or fever). Another word meaning inflamed was derived from the verb *napāhu*, "to blow": thus an inflamed finger would have been called a "blown finger." This peculiar expression becomes logical if one stops to consider that fire, in those days, was lit by twirling a fire-stick: a procedure which involved a lot of blowing to kindle the first sparks into a flame (the same notion of "inflating" is implied in the old term *spina ventosa* for long bones of the hand or foot slowly expanded by a mass of tuberculous tissue). In ancient Egypt, again, we find several words that can be translated as inflammation (e.g., *seref, shememet*). When these words are written in the original hieroglyphs, their meaning becomes obvious (or at least believable) even to the non-Egyptologist, because both words are followed by a special hieroglyph called a *determinative*. This sign was not pronounced, and served to convey the general idea of

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the preceding word. For *seref* and *shememet* it is a flaming brazier, symbolizing the notion "hot thing."

The Greek term for inflammation was *phlegmonê*, "the fiery thing" (phlox = flame). Ancient medical students must have wondered how this fiery condition could arise from *phlegm*, the cold and moist humor (whence we have the term *phlegmatic*). The contradiction was unexplained even in ancient times.

We will not bore the reader by repeating the Four Cardinal Signs of Celsus, but we do want to rectify the pedigree of the Fifth Sign, which was not added by Galen as the tradition holds. (Celsus, by the way, lived around the year 30 AD, and Galen between 130 and 200 AD: the dates are reversed in several textbooks [14]). It was L. Rather who first pointed out that Galen never added a Fifth Sign;[14] indeed, it would have been against his grain to do so, because he was so Greek at heart that he had little use for a Roman author, let alone for one who wrote in the language of the vulgar (Latin!) like Celsus. Throughout his monumental writings, he never even quotes Celsus. But who, then, originated the Fifth Sign?

It was, believe it or not, Virchow himself, in his *Cellular Pathology* (1858).[13,15] The episode is a magnificent example of the subtle, yet overwhelming influence that Virchow exerted over the world of pathology and over the thought processes of his peers. Virchow's statements had a way of becoming accepted as pure knowledge, as all-time truths. So, he dropped—just once—the remark that it was high time to add a fifth cardinal sign, the *functio laesa*, at least "according to the modern schools" (he does not explain which were these modern schools, but we suspect that "we, Virchow" was referring to his own "schools"). It took only a few years for the fifth sign to become a standard notion in Pathology texts; another few years later it metamorphosed, unaccountably, into an ancestral truth—promulgated by Galen.[13,14] This story is also an excellent example to show how medical legends arise and how textbooks carry them through, from generation to generation, unchecked.

Some Thoughts on Inflammation

The concept of inflammation and some of its satellite terms (such as acute and chronic) have come down to us from such remote antiquity that they carry with them, inevitably, a fair amount of vagueness; this tends to foster imprecise usage. We will, therefore, begin by defining some of the pertinent terms.

Most pathologists would probably agree that inflammation represents a response of living tissue to local injury; that it leads to the local accumulation of blood cells and fluid; and that the overall process, seen against the broad perspective of evolution, is a useful one, its primary significance being (in all likelihood) that of a defense against microscopic invaders. Good discussions on this overall issue can be found in the major textbooks of general pathology: those by Payling-Wright,[16] Pérez Tamayo,[17] and Florey.[18]

Inflammation and *injury* are often confused. By injury we will mean here the *passive* changes induced by a noxious agent. These changes may affect the cells, the extracellular materials, or both; from the injured area arise signals—chemical and perhaps also physical—which call forth the inflammatory reaction.

The twin terms *acute* and *chronic*, as applied to disease in general and to inflammation in particular, have now survived well into their third millennium; they are obviously practical and we can assume that they are here to stay. It is easy to use them in a loose fashion, especially in a clinical context, but more difficult to define their significance in terms of modern biologic science. In this regard, there are today two schools of thought. Some pathologists (probably most) firmly believed that acute and chronic inflammation represent two distinct aspects of the inflammatory reaction; others preach inflammation as a single entity, which cannot be split on the basis of its course in time. Our own view is that the ancient dual terminology is rooted in definite biologic (and histologic) events, and therefore should be retained; its use, however, is subject to a number of qualifications, as we will discuss below.

It is obvious that local injuries of all sorts induce an *immediate, acute* response which is basically the same whatever the agent; this immediate response is triggered by a variety of chemical "mediators" which can appear within the tissues in a matter of seconds and act primarily on the microcirculation, with two main effects: a) exudation of fluid, and b) exudation of white blood cells, primarily polymorphonuclear leukocytes (PMNs). This is, in essence, a *stereotyped, nonspecific response*. It is a beautifully planned mechanism of defense, with multiple pathways leading to similar useful effects, no matter what the cause.

If we go on to examine a typical focus of chronic inflammation, as caused by, for example, tuberculosis, we notice in the first place that it is teeming with mononuclear cells. Now we have learned that the somewhat dull histologic aspect of such infiltrates is quite misleading: few cells are as specifically informed, or programmed, as the lymphocytes and plasma cells (macrophages alone tend to retain, by and large, a nonspecialized function). We are therefore dealing with a highly specific response. (Only a few years ago, an infiltrate of mononuclear cells would have been called nonspecific: those were the days when little was known about lymphocytes and related cells except that they were more or less round, hence the glib term "round cell infiltrate").

Thus we may conclude that local injury can elicit two types of response: one immediate and nonspecific (Selye called it *local stress* [19]) the other delayed and highly specific. It seems quite proper to call them acute and chronic, even though these terms do not themselves convey the deeper biologic implication as regards their specificity.

All this being said, the following qualifications must be kept in mind:

1. The acute response, as defined above, *can* be brought about by an immunologic mechanism, but its principal characteristic is that it can be elicited by *any injurious agent*. This is in keeping with its evolutionary role of an all-purpose defense.
2. Although the acute response is, basically, stereotyped, it should not be construed as absolutely invariable; a slight degree of modulation, dependent upon the injurious agent, may be expected (see for example the eosinophilia of the immediate-type immune response).
3. The chronic form represents, in most cases, an immune response, i.e., a response to an antigenic substance; in these cases, lymphocytes and plasma cells predominate. *When the irritant is nonantigenic*, such as a lipid or an insoluble foreign body, *macrophages predominate* (with or without giant cells).
4. An acute response does not necessarily evolve into a typical chronic response, with a full complement of all types of mononuclear cells. A typical example is the allergic wheal (hives): here the acute response burns out without any chronic consequences, except for a few macrophages required to mop up the debris of the cellular exudate. A corollary (which was pointed out in 1960 by H. Movat [20]) is that *plasma cells do not belong to the normal course of aseptic wound healing*. The reason is obvious: antigenic challenge is minimal, and most of the mononuclear response is a matter of phagocytic removal by macrophages.
5. The oversimplified notion (favored by medical students who grope for firm rules) that polymorphonuclear leukocytes carry the message "acute inflammation," whereas mononuclear cells mean "chronic inflammation," contains some truth, but enough apparent contradictions to create classroom neurosis. One of these apparent exceptions is the *acute* formation of *lymphocytic* infiltrates, to be discussed below; another situation apparently fraught with anomaly is chronic purulent inflammation, as exemplified by osteomyelitis: what are the PMNs doing there, in a typically chronic context? The answer: *if the causative agent persists, and is chemotactic for PMNs*, the acute and the chronic responses are superimposed.

6. For the reason just mentioned, it is preferable to speak in terms of acute and chronic *responses*, rather than phases (because the latter word tends to imply on obligatory sequence, excluding an overlap).

7. The terminology of the immune response includes *immediate* and *delayed reactions*: how do these relate to acute and chronic inflammation? Here is another typical classroom question. The answer is that, in effect, the two sets of concepts overlap quite closely. The immediate-type immune responses (although the causal antigens are themselves very specific) trigger the routine, nonspecific inflammatory response; and the delayed responses are based upon the specific, mononuclear cell infiltrate of chronic inflammation.

8. *Viral infections* fit the least well in the overall scheme of acute and chronic responses. Thus, in many acute viral infections PMNs play a minor role because they are not attracted by viruses (in some cases, however, virus-infected cells have been shown to produce chemotactic agents for neutrophils and macrophages;[21] extensive, virus-induced necrosis may have a similar effect). Giant cells, so characteristic of chronic granulomatous inflammation, can form "acutely," in a matter of hours by the process of cell fusion,[22] such as in measles, and they can be epithelial as well as mesenchymal. Furthermore, a lymphocytic infiltrate can develop so rapidly as to compete with the classic acute response. It is good to keep in mind the experiments of Agus *et al.*,[23] who produced an acute viral gastroenteritis in human volunteers and found that the peripheral lymphopenia was paralleled by a heavy lymphocytic infiltration of the jejunal mucosa (with a few PMNs as well; see also Schreiber *et al.*[24,25]). *This infiltration developed within 48 hours of the oral administration of the inoculum;* it was interpreted as a redistribution of the lymphocyte population and may be a common feature of acute viral infections accompanied by transient lymphopenia. In other words, there exist lymphocytic infiltrates that develop "acutely"—as long as we accept a 48-hour event as "acute."

9. If we compare the clinical and the histologic use of the terms *acute* and *chronic inflammation*, it is obvious that *the histologic events evolve faster than the clinical picture*. A heavy acute infiltrate may be present histologically prior to the appearance of clinical symptoms; and after 4 or 5 days a boil may look extremely acute clinically, whereas by that time the histology already includes a major "chronic" component.

Vascular Events

Two sets of vascular events take place in acute inflammation: changes in flow and caliber, and changes in permeability.

Changes in Vascular Flow and Caliber

These are of primary importance in the development of the acute inflammatory reaction, because they determine—to a large extent—the amount of exudate. If local blood flow is decreased or temporarily stopped, exudate will be reduced or abolished. It is common laboratory experience that if an animal is under deep anesthesia (and especially if the skin is allowed to become cold) the local permeability-increasing effect of histamine-type substances becomes difficult or impossible to demonstrate by the usual vascular labeling techniques: the blue or black spots fail to develop as expected because the blood supply to the area has failed. An extreme example of this pathophysiologic mechanism is offered by shock, which implies, by definition, inadequate tissue perfusion: if rabbits are made hypovolemic by removal of 30 to 40% of their blood volume (causing a blood pressure drop to 70% of normal), the Arthus reaction is inhibited, and the accompanying edema is markedly diminished.[26]

The mechanisms responsible for the control of local blood flow in inflammation have not attracted much interest since the studies of Lewis and his collaborators, which led to the definition of the triple reaction.[27] Perhaps the most significant advance was the demonstration that the flare after histamine injection is, to some extent at least, under the control of the central nervous system. In a study of 75 patients with cerebrovascular lesions, it was shown that disturbances of sensation, in the presence of sensorimotor cortex lesions of one side, were associated with enhanced flare on the contralateral side of the body (the diameter of the flare was 68.3 ± 9.5% larger, $P < 0.001$); thalamic or spinothalamic lesions were associated with a decreased flare on the contralateral side (flare diameter was 36.0 ± 14.5% smaller, $P < 0.001$). In 2 patients with clinically complete transection of the spinal cord, the flare was an average of 24.5% smaller in anesthetic dermatomes of the thigh. Paralysis alone without sensory deficit did not alter the size of the flare.[28] More work is needed along these lines.

There have been several attempts to explain the vascular leakage of inflammation on the basis of pure vasomotor effects. Thus, it was stated that certain chemical mediators of inflammation induce a contraction of the small veins, whereby the small venules upstream are stretched until their endothelium comes apart at the seams.[29] This theory seemed attractive because it correlated with the fact that substances which induce venules to leak are also capable of inducing smooth muscle to contract (e.g., histamine, bradykinin). However, cinematographic studies on the rat cremaster muscle and mesentery have shown that the alleged venous contraction does not occur after the local application of histamine-type mediators, and therefore it cannot be considered a key mechanism.[30,31] The double action of smooth muscle stimulants—on smooth muscle contraction, as well as on venular permeability—turned out to have a different and equally attractive explanation: it is true that the permeability effect is caused by cellular contraction, but the contractile response occurs at the level of the endothelial cells (see further).

Changes in Vascular Permeability

The notion of *increased vascular permeability* should have had its centennial celebration 4 years ago. When Cohnheim wrote his first and best-known paper on inflammation in 1867,[32] he described diapedesis as well as changes in vascular caliber and flow, but he never mentioned an increased permeability of the blood vessels. At that time it was believed that there were small openings along the interendothelial junctions—"stomata" and "stigmata"—said to be visible with the silver nitrate technique; Cohnheim felt that the extravasation of cells and fluid occurred across these normal openings, slightly stretched by the dilatation of the blood vessels. The notion of increased permeability appears for the first time, as an afterthought, in a lengthy paper published by Cohnheim 6 years later.[33] Here his supporting evidence was fair, but indirect; oddly enough, he did not try to clinch his point by injecting intravenously either a dye or a colloidal pigment to visualize the leakage. Yet both laboratory techniques were very simple and familiar to him. He used intravascular injections of dyes in his studies of embolism and infarction. He even injected colloidal pigments for labeling phagocytes in inflammation. Perhaps, in these experiments, the leaking vessels were also labeled; but Cohnheim was looking for labeled cells and may have disregarded the labeled vessels as a nuisance. It is even more amazing to consider that an eminent anatomist, Julius Arnold, was injecting colloidal suspensions of pigments at that very time.[34] Arnold did most of his experiments on frogs, in which the small vessels are unusually permeable under normal conditions; whenever he saw a deposit of pigment in the vascular wall, he interpreted it as an indication of local leakiness: hence he was using precisely what we now call the technique of *vascular labeling*, our most useful method for the study of leaking vessels. He even injected a mixture of blue dye and gelatin for the specific purpose of demonstrating "puffs" at leaky points! Why Cohnheim never took these obvious hints from Arnold is impossible to tell; one reason may have been that Arnold was studying normal blood vessels—and pathologists have a notorious tendency to disregard the normal as if it were nonexisting.

It is already apparent from this introduction that vascular labeling is an important tool in our understanding of vascular leakage. We will therefore explain the method briefly; details can be found elsewhere.[35,36]

The technique of vascular labeling is a simple morphologic device whereby those vessels of the microcirculation which have become leaky without being totally disrupted can be individually identified by light as well as electron microscopy. It is possible to label a leaky vessel if its endothelial barrier alone is interrupted, whereas the underlying basement membrane remains intact. These conditions prevail quite often because the basement membrane—although much thinner than the endothelium—is surprisingly tough; also, it is not very firmly attached to the endothelial cells, so that the latter can pull apart from each other and even slough off without tearing apart their basement membrane.

In a microscopic vessel which has become leaky in this manner, flow is still possible, and blood cells float by without escaping: but plasma seeps out across the basement membrane, which behaves like a coarse filter. Thus, much protein is lost, while some of the larger molecules (especially lipoproteins) are retained, together with larger particles such as chylomicrons. Colloidal materials injected intravenously are also retained if the particles are of appropriate size. The particles trapped against the basement membrane are retained in the wall of the vessel for days, weeks, and even months.[37] It is, therefore, appropriate to define these vessels as "labeled." Eventually most of the deposits are phagocytized by the endothelium and the pericytes. The handiest colloidal suspension for such studies is India ink (carbon black, particle size approximately 300 Å), which has the great advantage of being easily visible by light as well as by electron microscopy. A special variety of India ink, prepared without shellac, is commercially manufactured by the Pelikan Company.[38]

Bluing, by contrast, is a method whereby an area of increased permeability can be demonstrated, but without visualizing the individual leaking vessels. A dye such as Evans blue or trypan blue is injected into the blood stream where it becomes immediately bound to the serum albumin. Wherever there is a leak, the dye–albumin complex seeps out and forms a blue patch; being too small to be retained by the basement membrane it causes no discrete labeling of individual vessels. Despite this drawback, the method is very useful because it is a sensitive indicator of increased permeability; furthermore, the blue dye is easily quantitated to allow comparative studies of the potency of different mediators (see Wilhelm[39]).

Direct and Indirect Vascular Injury

It is essential to realize that vascular leakage, after local injury, can occur by at least two distinct mechanisms: a) directly, as an effect on the injurious agent itself (heat, mechanical trauma, etc.) or b) indirectly, as an effect of chemical substances that appear in and around the site of injury. This basic distinction is already hinted in Cohnheim's paper of 1873 but was not clearly stated until comparatively recently.[40,41] One may wonder why this very obvious fact was so slow to be recognized. The reason is simple: there was little point in distinguishing direct and indirect injury (except as abstract concepts) unless a method was available to distinguish one from the other. The technique of vascular labeling made this possible: it showed, in essence, that direct injury can affect all types of vessels (arterioles, capillaries, and venules), whereas indirect injury shows a high degree of specificity for the venules.[35] The difference between these two patterns can be strikingly demonstrated by means of experimental burns of the skin: if the thermal injury is appropriately calibrated, the underlying muscle will show a central patch in which all vessels are labeled (by direct injury). This is surrounded by a halo in which only the venules are labeled (an expression of mediated injury).[7,40,41]

The distinction between direct and indirect injury is of practical importance because the pharmacologic means to affect one or the other may not be the same. However, it must be kept in mind that this distinction—theoretically so simple—is not always a sharp one. In the so-called delayed form of vascular leakage (to be discussed below), it was long debated whether the mechanism is direct or indirect. Furthermore, it is becoming increasingly obvious that bacteria can produce substances similar or identical in their effects to chemical mediators of inflammation produced by injured tissues; hence, it is quite conceivable that a directly injurious agent, such as a bacterium, may induce inflammation by borrowing an "indirect" pathway. The very name of *pyogenic* bacteria indicates that some bacteria can produce substances chemotactic for polymorphonuclear leukocytes; filtrates of cultures of various bacteria have chemotactic activity for neutrophils.[42] Lymphokine-like activities have also been found in supernatants of *Escherichia coli* cultures.[43] Permeability-increasing products of the histamine type—as far as we know—have not yet been described. They would be of no known help to the bacteria, indeed, they would have suicidal value by promoting inflammation; but then, also the leukotactic factors are suicidal. The lesson that emerges here is that bacteria have not yet learned, through evolution, that by helping inflammation they help dig their own grave.

Ultrastructure of Vascular Leakage

If we accept the basic notion that the vessels of the microcirculation can be induced to leak by two major pathways—direct and indirect—we must proceed to examine the cellular mechanisms of such leakage. It is now well established that the main permeability barrier (perhaps in all but the glomerular vessels) is the endothelium. We can, therefore, expect that most of the pertinent cellular events will lie beyond the resolution of light microscopy (the single exception being silver impregnation of the inter-endothelial junctions, which can provide important light microscopic data on the endothelial layer).

Electron microscopy has opened new vistas, but despite this advance, it should not be forgotten that to this date, early 1976, even the normal mechanism of transendothelial exchange is not entirely settled. Furthermore, in the field of pathology, the standards of electron microscopic technique are allowed all too often to slip, with the unwritten excuse that poorly fixed and poorly processed tissues owe their distressing looks to disease.

As regards the normal mechanism of transendothelial exchange, two recent contributions—both from Dr. Palade's laboratories—need to be emphasized. First, some endothelial cells appear to be traversed by short channels of the diameter of a pinocytic vesicles; these channels are usually too tortuous to be seen in a single section, and were demonstrated by examining single ultrathin sections at various angles ("tilting").[44] Second, the intercellular junctions in venules are not as tight as in other small vessels; this was demonstrated by freeze-cleaving of mesenteric arterioles, capillaries, and venules [45] (see also Addendum).

Among the cellular mechanisms that may cause the endothelial layer to leak, the following have been demonstrated or suggested:

Endothelial Destruction. This is self-evident; the cells are broken up or dead, whereas the basement membrane remains. When this occurs, platelets adhere to the damaged surface, but thrombosis does not usually proceed to obliterate the lumen.[46-49] Endothelial death may occur with a considerable delay after exposure to the noxious agent (see further).

Formation of Intercellular Gaps by Endothelial Contraction. Histamine-type mediators induce ultrastructural changes [50] highly suggestive of endothelial contraction in the venules.[51,52] These changes include: a) bulging of the cell body into the lumen, b) a change in shape of the nucleus, from oval to rounded, c) the appearance of multiple folds in the nuclear membrane, including very tight, "pinched" folds, d) the appearance of similar folds in the cell membrane, usually on the abluminal surface, e) the presence of bundles of filaments, 40 to 70 Å in diameter and sometimes marked by darker bands arranged periodically, f) the appearance of an intercellular gap on one or both sides of the endothelial cell, as seen in an ultrathin section. When two adjacent cells pull apart from each other, they often remain partially connected by long, thin branches of cytoplasm which remain taut as cords across the gap (see Majno and Palade,[50] Figures 7 and 8). Similar thready links have been observed between endothelial cells cultivated *in vitro* (see Blose and Chacko,[53] Figures 8A and B, 9A). Thus it is not uncommon to see, in an ultrathin section, a red blood cell slipping out through a gap while being cut in two over such a taut cord, like a butter being cut with a string. These branches of endothelial cells, running across a gap from one cell to another, have given rise to several misinterpretations of electron micrographs: it was said, for example, that the gaps occur inside cells, rather than between them,[54] or that these structures represent a "bubbling" of the endothelial cell surface whereby the cells would come apart without contracting [55] (in fact, there is no such bubbling on the free surface of the endothelium as a result of histamine-type drugs).

The notion that venular endothelium is contractile, suggested at first by purely structural electron microscopic evidence, was further supported by the demonstration that endothelial cells contain "contractile protein" [56,57] and by the demonstration of endothelial contraction *in vivo*, in venules of the rat mesentery.[58] On the other hand, we are not at all convinced that endothelial contraction can occur also in large arteries.[59] Furthermore, it should be kept in mind that the mere presence of intracellular filaments cannot be equated with cellular contraction; while two classes of myofilaments, "thin" and "thick" (40 to 70 and 170 Å, respectively), have been equated with actin and myosin, there is a class of 100-Å filaments which have not been correlated with any muscular protein and may be semirigid structures, functioning as a sort of cytoskeleton.[60]

Formation of Gaps by "Unzipping" of the Endothelial Junctions. When tissues are examined after relatively mild injuries, as produced by heat [47,61] or turpentine and nitrogen mustard,[61] leaks often appear between endothelial cells; and the latter appear sometimes normal, sometimes darker [61] or with other evidence of damage. The overall impression is that such gaps can occur also as a result of mild direct injury (i.e., without endothelial contraction), as if the intercellular junction were particularly delicate. Acute cadmium intoxication brings about intercellular gaps without evidence of contraction;[62] emigrating leukocytes, too, are able to cleave endothelial cells apart. The evidence available suggests that the unzipping phenomenon is real; it may play an important role in vascular pathology.

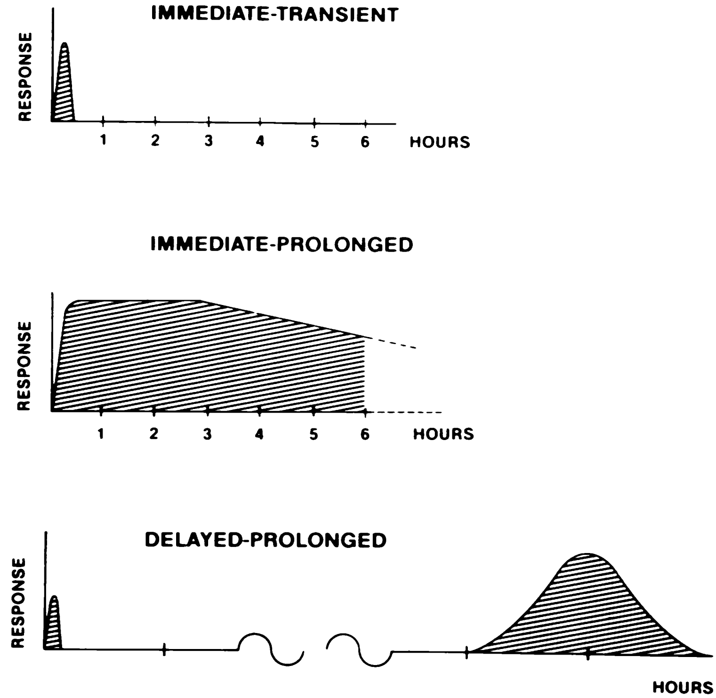
Transcellular Leakage. In theory, it seems unlikely that a living endothelial cell could give up its basic right to a private *milieu intérieur*, become freely permeable, and allow itself to be soaked through by plasma. Yet there are two reports stating that this may occur in lung capillaries under the effect of anthrax or cholera toxin.[63,64] Further studies are needed before this can be recognized as a fact. However, the notion of transcellular passage becomes acceptable if it is intended as an expression of *transcellular canals;* since it now appears that such canals exist in normal endothelium,[44] it is quite conceivable that they could increase in size and/or number under pathologic conditions. This problem is not yet settled.

"Increased Active Transport" by the Pinocytic Vesicles. Descriptions of endothelial cells in inflammatory lesions often include the statement that there is "increased vesiculation." This peculiar term remains, for the time being, an expression of wishful thinking. Sometimes an increase in diameter of the vesicles, as an expression of pathologic swelling, brings about an apparent increase in number, but even if the number of vesicles were really increased, this would be no proof of increased transport.

Other Possible Sources of Inflammatory Exudate. In the first place, it should not be forgotten that exudate is always mixed with transudate: arteriolar dilatation causes the pressure in the microcirculation to rise, and this will bring about an increase in ultrafiltration across all those endothelial surfaces that have remained intact. Furthermore, the lesson of cholera toxin has taught us that intestinal epithelial cells can produce enormous amounts of fluid if a toxic agent interferes with their normal secretory mechanism.[65,66] Secretion can also be a prominent activity of connective tissue cells (especially of fibroblasts). It is, therefore, quite possible that bacterial or other products may irritate local cells and induce them to secrete excessively or abnormally. The first steps taken in this direction are intriguing: several human tissues were found to contain a *connective tissue-activating peptide* which induces hypermetabolism in normal fibroblasts in tissue culture: hyaluronate and lactate formation were increased and glucose uptake was also increased, whereas the formation of soluble and fibrous collagen was depressed.[67] This example of cellular irritation is highly reminiscent of Virchow's concept of *parenchymatous inflammation.*[68]

Functional Patterns of Vascular Leakage

Thus far we have analyzed the various cellular mechanisms that may contribute to the pathogenesis of vascular leakage. If we now examine the phenomenon of vascular leakage from the functional (clinical) point of view, as it develops in time, we find that it can present itself according to one of three basic patterns, or a combination of these (Text-figure 1). The next step must therefore be to describe these functional patterns and attempt to correlate them with the structural events. Excellent reviews of



TEXT-FIGURE 1—Types of vascular leakage that may occur in a focus of injury classified according to their course in time. The delayed-prolonged variety (*bottom*) is often preceded by a short burst of leakage of the immediate-transient variety.

the pertinent literature may be found in two recent Australian contributions: Hurley's monograph on acute inflammation [7] and Wilhelm's chapter on chemical mediators, in Volume II of *The Inflammatory Process*.[39] We will propose, however, a slightly different terminology, as explained below.

Immediate-Transient Leakage. Immediate-transient leakage (Text-figure 1, top) is a burst of leakage that occurs immediately after exposure to the injurious agent and lasts for a very short time; its average duration is of the order of 15 to 30 minutes. It will occur *within* the traumatized area if the damage is slight (good examples are the allergic wheal or the wheal of the triple reaction as brought about by stroking the skin with a blunt object) and *around* the affected area if the tissue is destroyed. Structurally, it is characterized by its high degree of specificity for venules up to about 100 μ in diameter (and therefore not yet provided with smooth muscle cells). It is firmly established that this response is due to chemical substances liberated in the injured area; it can be reproduced, with its typical features—prompt appearance, short duration, and venular topography—by the local injection of histamine-type mediators.[7,40] These are a chemically disparate group, hence, it is not surprising that antihistamines alone will not always suppress this response.[7] Of the three patterns of leakage, only this one is definitely known to be caused by specific, endogenous chemical agents; thus, it should also be (in theory at least) the most susceptible to pharmacologic inhibition.

In the terminology of Wilhelm,[39] the response described above is referred to simply as "immediate;" we prefer the clumsier but clearer term *immediate-transient*, to avoid confusion with the next variety, which is also immediate but not transient.

Immediate-Prolonged Leakage. Immediate-prolonged leakage (Text-figure 1, middle) is the typical result of direct injury: it starts immediately and continues until the damaged vessels are repaired or plugged, even as long as 2 days.[7,40] A good example is the leakage that follows a severe burn.[69] Little is known regarding the manner in which the injured vessels are repaired. Presumably, new endothelial cells crawl along the basement membrane, wherever it is preserved, and perhaps also take part in the cleaning-up process by phagocytizing cell debris and other extraneous materials. (This variety of leakage response has been referred to as "early;"[39] we find this misleading because the histamine-type response described above is just as "early.")

Delayed-Prolonged Leakage. In the case of delayed-prolonged leakage (Text-figure 1, bottom), it is easy to describe the functional events, but not their pathogenesis. The initial observations were made at the Lister Institute by Sir Ashley Miles and his collaborators. In testing guinea pig skin by the bluing technique, after the local injection of live bacteria or of *Clostridium perfringens* alpha toxin, they found an initial short burst of increased permeability (of the type referred to above as immediate-transient), followed after 2 to 4 hours by a second and more persistent episode.[70] A similar delayed-prolonged reaction was observed by Sevitt in 1958, using a mild experimental burn (55 C for 5 seconds).[69] Since then, delayed reactions have been described with a number of injurious agents, including cold, x-rays, ultraviolet rays, bacterial products, toxins, and a variety of chemicals including kaolin,[71] carrageenin,[72] and, of course, turpentine (see Hurley[7] Wilhelm[39]). A good model for studying this phenomenon is mild thermal injury produced with the flat end of a copper cylinder maintained at the desired temperature with circulating hot water. Using this method on the skin, and by combining it with the techniques of vascular labeling, it was found that the leakage occurred overwhelmingly in the superficial capillary network;[41] a predominantly capillary lesion was also described in the rat cremaster muscle when the hot copper surface was applied to the scrotum.[73] Thus, it was concluded—

at that time—that the delayed prolonged leakage affects mainly the capillaries. It was, as we shall see, a premature conclusion; those who adopted it, including one of us, were perhaps affected by the human—if not scientific—temptation to accept comfortable symmetries ("the immediate response is venular, the delayed is capillary"). It was also suggested that this capillary lesion was probably due to specific chemical mediators. This, too, was premature.

At that time, two experimental features of the reaction were especially puzzling: a) the predilection for the capillaries and b) the mechanism of the delay. Satisfactory explanations for both have now been provided by Hurley and collaborators, as we shall discuss below.

First, the problem of selectivity for capillaries. It was certainly true that the lesions described in two models were mainly capillary, but this could also be explained—for the skin especially—by the anatomic location of these vessels: being closest to the surface and, therefore, the damaging agent, they are also the most susceptible to show the first signs of injury. If this is true, then the same type of heat injury applied to a microvascular network with a different pattern should show a different selectivity. This prediction was correct: by applying the heated disc directly to the fascia of the rat's abdominal muscles, where the pattern includes both capillaries and venules, the delayed reaction took place in both kinds of vessels.[74]

Next, the problem of the delay. To explain it, Ryan and Hurley [75] began by drawing an analogy between heat injury to the microvascular endothelium and toxic injury to liver cells. It was already an established fact that carbon tetrachloride, for instance, reaches the liver several hours before structural evidence of damage can be demonstrated: this suggested that a similar phenomenon could take place in vessels damaged by mild heat. To test this hypothesis, Ryan and Hurley selected a number of drugs capable of protecting liver cells against toxic injury (such as promethazine) and found that they also suppressed the vascular leakage induced by mild thermal injury. This was a further argument to suggest that the delayed leakage is due to direct injury of the endothelium. Yet another argument is the following: if the intensity of the stimulus is increased (i.e., with a more severe burn) the delay becomes shorter and the reaction eventually becomes monophasic (i.e., immediate-prolonged).[7]

A third feature of the delayed response that needs to be explained is its prolonged nature. This, too, becomes easy if we are dealing with direct injury; in lesions produced with mild heat, Ham and Hurley [76] found gaps resembling those caused by histamine, but also larger gaps accompanied by obvious endothelial damage (and more severe after 24 and 48 hours than after 2 hours).

We believe, then, that the delayed-prolonged response is, to a large extent, a special case of direct injury—more precisely, a consequence of mild direct injury. We qualify this by adding "to a large extent" because there is still the possibility that mediators may play a lesser role: perhaps special long-acting mediators,[77-80] perhaps also short-acting but continously released agents. Studies are urgently needed to determine the chronic local effect of histamine-type mediators. But whatever studies on the pathogenesis of delayed reactions might show, it is already clear that a specific substance, effective only on the permeability of capillaries, is not required.

We would like to add that the notion of "capillary mediator" has several strikes against it, anyway, on purely theoretical grounds. Nature seems to have been highly protective toward the capillaries. First, in the evolution of the acute inflammatory reaction, as it occurs after mechanical trauma, it is obvious that many alternate pathways (see section on Mediators) were developed to make sure that the venules would leak—and yet none of these chemical mediators, as far as we know, has been shown to affect capillaries. A second example of "capillary sparing" is offered by diapedesis: leukocytes unfailingly float past the capillaries and choose to stick and emigrate only when they reach the venules. Thirdly, apart from diabetes, capillaries seem to be among the safest tissues in the body; even old age leaves them very nearly intact. They have almost no diseases of their own.

The venules, instead, have a long record as targets of disease; leakage, diapedesis, petechial bleeding, allergic injury, thrombosis, all have a morbid predilection for these vessels. Why? Is this merely an accident, perhaps related to the looser junctions of the venules?[45]

If we may be allowed a final speculation on this topic, we believe that Nature has strong reasons for sparing the capillaries, the real functional units of the entire vascular system. It has arranged matters in such a way that the precious capillaries will always continue to function, blindly, without much freedom of choice; the flexibility of control being assigned, upstream, to the arterioles. If this were not so, imagine what would happen if diapedesis, for instance, occurred in the capillaries, where the lumen is so narrow that leukocytes have to squeeze their way along one by one. As soon as the first leukocyte came to stick, as a necessary first step of diapedesis, it would have the effect of an embolus: the circulation through that capillary would come to a complete stop and would not resume until the leukocyte had managed to work its way out across the wall, which takes several minutes. This would be tantamount to stopping the circulation at a time when a brisk circulation is needed. It is also possible that the embolized leukocyte might run into a behavioral problem: wedged in the lumen, like a cork in a bottle, with endothelium all around—a junction along its left side and another one along its right—might it not be tempted to send our a pseudopod through each of the junctions and then remain caught in the middle? It is not uncommon to see red blood cells behave in this manner, i.e., letting their soft bodies passively stream out through two adjacent endothelial gaps. Finally, if histamine-type mediators caused the capillaries to leak, instead of the venules, a circulatory problem would arise: the loss of plasma would rapidly cause the red blood cells to become packed into a column, which would encounter a much greater resistance to flow. A similar packing phenomenon does occur also in the venules (it is included in the term *stasis* as physiologists use it), but here the ratio of plasma volume to endothelial surface is greater, so that total loss of plasma and complete packing are less likely; flow is therefore likelier to continue.

For these reasons, we feel that the discovery of an endogenous mediator specifically affecting the capillaries is improbable. After all, we do not yet know a single pharmacologic agent capable of modifying the physiologic functions of the capillary wall. Perhaps Nature has deprived the capillary endothelial cells of appropriate receptors—to preserve them from the temptation of responding.

Cellular Events (Leukocytic Infiltration)

The pathognomonic histologic feature of inflammation is the infiltration by leukocytes. In the early stages, and especially if there is bacterial infection, the predominant cell is the neutrophil. In the later stages, and during resolution, the predominant cell is the mononuclear phagocyte. Both of these cell types are derived from the corresponding blood cells which stick to the endothelium and emigrate across the vessel wall. Once in the tissues, the leukocytes crawl about and phagocytize bacteria and cellular debris. Recent studies of the clinical defects of leukocytic function have shown clearly that disorders of cellular locomotion, chemotaxis, phagocytosis, and/or microbicidal activity can lead to serious, sometimes lethal, susceptibility to infection.

Leukocytic Sticking

Pavementing, or sticking, of leukocytes against vessel walls in inflamed areas occurs mainly in venules.[81] An early idea attributed this sticking phenomenon to the presence of a glue-like material on the luminal surface of the endothelium [82] and indeed, using special staining techniques (e.g., ruthenium red,[83] colloidal iron,[84] and alcian blue [85]), a cell coat material has been demonstrated inside all blood vessels. No significant alteration in this layer has been observed in inflamed vessels,[7,84] but it is not certain that an increase in the stickiness of the layer would necessarily be associated with a detectable morphologic change. A different view was taken by Swedish workers [86] who studied inflamed rabbit mesentery and the hamster cheek pouch and concluded that wherever a granulocyte (or a red blood cell or platelet) becomes stuck, it is always overlying an endothelial gap 0.1 to 1 μ in diameter or larger; these gaps were said to "bear no relation to the endothelial junctions" (the latter statement is rather surprising, because it cannot be made on the basis of electron microscopy alone). Endothelial gaps may certainly trap cells of all kinds, but leukocytic sticking is an entirely different matter, as proven by the "rolling" of marginating leukocytes along the endothelium. Attempts have also been made to explain this stickiness by an invisible, molecular change; it is possible that a local alteration of the cell membranes somehow permits calcium-bridging between the endothelium and the leukocyte; Bangham [87] has suggested that this bridging might commence at sites of contact between cell protruberances (e.g., pseudopods), i.e., at sites where mutually repulsive electrostatic forces would be reduced. That divalent cations, such as Ca^{2+}, might play a key role in leukocytic sticking is supported by the finding that chelation with EDTA *in vivo* prevents pavementing in the rabbit ear chamber [88] and in the hamster cheek pouch and mouse mesentery.[89] In addition, local anesthetic agents suppress pavementing *in vivo;* [90] such drugs inhibit membrane depolarization by reducing sodium and potassium conductances [91] apparently following displacement of Ca^{2+} from binding sites in the membrane,[91-94] but they are also known to induce physical "stabilization" of cell membranes [95] and to prevent the redistribution of membrane-bound molecules.[96] In summary: All that we can say about the mechanisms involved in leukocytic pavementing *in vivo* is that divalent cations may be somehow implicated.

In line with the current interest in examining human leukocytic function, methods have been devised for quantifying leukocytic stickiness. These methods depend upon the adhesion of neutrophils to glass surfaces [97,98] or nylon fibers [99] and are thus open to the objection that the findings may bear no relationship to the sticking of leukocytes to endothelium. Despite this, the results so far indicate that the approach should prove useful. For example, it has been shown that adhesion depends upon glycolysis and requires the presence of divalent cations [97] and is suppressed by agents that increase intracellular cyclic AMP (e.g., dibutyryl cyclic AMP, prostaglandin E_1, histamine, theophylline) [100] or by treatment of the cells with ethanol, prednisone, or aspirin.[99]

Leukocytic Emigration

As foretold by Arnold [101] in 1875, electron microscopic studies have shown that leukocytes migrate across the vessel wall via the interendothelial junctions.[102,103] The claim by Marchesi and Gowans [104] that lymphocytes traverse endothelial cell cytoplasm in lymph node postcapillary venules has been refuted by Schoefl [105] and by Wenk *et al.*; [106] using careful serial sectioning techniques, the latter two groups found that lymphocytes which appear to be intraendothelial can, in fact, be demonstrated to lie within the junctions. Hurley [7,107,108] has reported that leukocytic emigration alone (i.e., in mild injury) does not induce vascular leakage, indicating that the leukocytes must insinuate themselves tightly down the junctions. Neither does leukocytic emigration enhance the local accumulation of ^{131}I-labeled albumin at sites of injection of histamine.[109] However, if vascular leakage is provoked (e.g., by injecting histamine) concurrently with leukocytic emigration (e.g., in a skin site injected 4 hours previously with serum), intravenously administered carbon particles escape freely into the extravascular tissues, producing irregular, blotchy blackening.[110] This apparently occurs because the basement membrane surrounding the venules is temporarily disrupted by the escaping leukocytes.[7,110] Leukocytic sticking and migration across the endothelium can also occur in medium-sized veins as a response to perivascular trauma.[111]

The mediation of leukocytic emigration is uncertain. From histologic studies of rat skin following the injection of various agents, Hurley and Spector [112,113] concluded that factors responsible for leukocytic emigration are produced when a serum substrate is acted upon by substances derived from injured tissues or neutrophils. As discussed later (see section on Chemotaxis), it was subsequently demonstrated that such factors may also have chemotactic activity.[114-117] It is possible that chemotactic agents seep into interendothelial junctions and thereby attract pavemented leukocytes, but there is, as yet, no evidence for this concept.

Time Course of Leukocytic Infiltration and Cell Types Involved

The number of cells migrating into inflammatory exudates can be measured following the injection of phlogistic agents into the peritoneal cavity,[118] the pleural cavity,[119] or into chambers placed over minor skin abrasions.[120] The findings show that, with relatively mild irritants (e.g., serum and glycogen solutions), neutrophil accumulation reaches a peak at approximately 4 hours and then declines rapidly, whereas the total number of mononuclear cells begins to rise only after 4 hours and reaches a sustained peak at 18 to 24 hours.[119] The height of the neutrophil peak shows great variation with different stimuli, whereas little variation is found in the mononuclear peak.[119] When living *Klebsiella pneumoniae* microorganisms are injected intrapleurally, massive levels of neutrophils develop and rise progressively throughout the 24 hours of observation; no mononuclear response can be detected in this period.[119] It was previously suggested that neutrophils and mononuclear phagocytes migrate simultaneously into inflammatory exudates, and that the neutrophils rapidly disappear, thus exposing the less numerous, but more long-lived, mononuclear cells at the later stages.[121] This was supported, at the time, by the belief of Harris [122] that neutrophils and monocytes showed no differences in chemotactic reactivity to various stimuli. However, the results of the experiments just described indicate that the entry of monocytes into the exudates starts as neutrophil emigration declines and continues for a considerably longer period. Furthermore, as discussed in the section on Mediators (see Table 12), certain differences in chemotactic responsiveness have since been discovered for the two cell types. For instance, monocytes apparently respond significantly to a neutrophil-derived lysosomal cationic protein.[123]

Chemotaxis

Chemotaxis, the attraction of cells towards chemical substances, has fascinated biologists for nearly 100 years (see reviews by Ramsey and Grant [124] and Wilkinson [125]). By the 1940s, largely from the work of Leber,[126] Commandon,[127] the Clarks,[128-130] and McCutcheon's group,[131-133] it was generally believed that neutrophils were attracted by various bacteria and their products and by factors released during tissue breakdown. However, in the early 1950s, Harris [134,135] proposed that much of the data concerning leukocyte chemotaxis were derived from poorly controlled experiments. Particularly justifiable was his claim that previous experimental models often failed to distinguish whether cellular accumulations were due to true chemotactic attraction or were simply explained by trapping of randomly moving leukocytes. Harris [134] therefore devised a system which allowed direct observation and continuous photographic recording of leukocytic locomotion; the cells were incorporated into a film of clotted plasma between a slide and coverslip and, using dark field microscopy and long exposures of the same photographic plate, the paths traced by the cells were seen as white tracks on a black background. Harris confirmed that clumps of various bacteria were chemotactic; he also found that there was no polarization of the tracks towards fragments of injured or autolyzed tissue.

The next important development was the introduction of a new assay system by Boyden [136] in 1962. Boyden's system consists essentially of a chamber with two compartments separated by a horizontal filter membrane; leukocytes placed in the upper compartment crawl through the pores of the filter when a chemotactic solution is placed in the lower compartment; the chemotactic activity of the fluid in the lower compartment is evaluated by counting the number of cells on the lower side of the filter at the end of a certain time. In a later modification of the technique, Keller *et al.*[137] placed a second, less porous, filter beneath the cell-permeable filter to catch and count cells that might detach from the lower surface. The advantages of the Boyden system are that it can be used to assess the chemotactic activity of substances in solution, and that it allows some degree of quantitative comparison of the chemotactic activity of different test substances. The major, and indeed crucial, disadvantage is that, as ordinarily performed, it may not distinguish between accelerated random locomotion of cells and true chemotaxis. Boyden himself recognized this problem and performed control experiments in which the chemotactically active solution was placed with the cells in the upper compartment of the chamber; in such cases, the cells showed less tendency to crawl into the filter membrane than if the solution was only placed in the lower compartment. This was interpreted (and repeatedly quoted) as evidence that accelerated random locomotion was not involved in a positive result. However, this assumption has been critically reexamined by Zigmond and Hirsch,[138] who found that substances may stimulate locomotion in low concentrations but may inhibit locomotion at higher concentrations. These workers concluded that "under most circumstances it is difficult or impossible to distinguish in the Millipore (Boyden) system between effects on locomotion or chemotaxis." They proposed a more rigorously controlled approach whereby the distance moved by the cells into the filter was first determined in the absence of a gradient but, in individual experiments, with a full range of concentrations of the test material above and below the filter. These results were then to be compared with those obtained using a full range of gradients, thus enabling an analysis to be made as to whether cells exposed to a positive gradient moved into the filter more rapidly than expected on the basis of random locomotion alone, i.e., whether the cells were indeed responding chemotactically. This sounds well in theory, but it is obviously time-consuming and it is still likely that differentiation between stimulated random locomotion and true chemotaxis in this system will remain "exceedingly difficult." [139] We agree with Hirsch [139] that results obtained with such indirect techniques should be checked by direct microscopic observation of leukocytes crawling in thin preparations, such as that used by Harris.[134]

Having evaluated the technique, let us now return to Boyden's original observations. He discovered that chemotactic activity (for neutrophils) was generated from a heat-labile serum system (presumably complement) during incubation with antigen-antibody precipitates.[136] Hurley [114] soon utilized the technique to show that serum activated by minced tissue was also chemotactic. Ryan and Hurley [115] then used Harris' [134] photographic trace method to directly demonstrate that neutrophils crawled toward tissue fragments which had been previously incubated with fresh serum. These findings not only reinstated the view that chemotactic factors can be released during tissue damage but also established the new concept that such factors may be derived from an interaction between tissue product(s) and serum substrate(s). As detailed later (see section on Mediators), many investigators have extended this work and pinpointed the key role of serum complement in the production of endogenous chemotactic factors (e.g., C3 fragments and C5 fragments) (see review by Ward [140]).

As well as giving rise to products which release chemotactic molecules from complement components, it appears that injured cells may also liberate serum-independent chemotactic agents. Using a slide-coverslip system, Bessis and Burté [141] injured individual cells by means of a laser beam and directly observed attraction of surrounding neutrophils towards the damaged cells; Bessis [142] coined the term *necrotaxis* to describe this phenomenon and stressed that attraction occurred for only a short time, during disintegration of the target cell. It was subsequently found that this attraction occurred in a serum-free medium.[143] Grimes and Barnes [144] suggested that cyclic AMP may be the chemotactic principle released from such laser-injured cells. From the results of slide-coverslip experiments, Zigmond and Hirsch [138] proposed that damaged or phagocytizing neutrophils may liberate serum-independent chemotactic products, possibly lysosomal in origin (see section on Mediators).

Studies using the Boyden system have shown that leukocytes other than neutrophils, viz. mononuclear phagocytes, eosinophils, basophils and perhaps lymphocytes, can respond chemotactically to various stimuli.[140] These stimuli are discussed later (see section on Mediators and Table 12).

What are the mechanisms involved in chemotactic responsiveness? First, how do leukocytes detect a gradient of chemotactic molecules? And secondly, how does sensing of this gradient trigger directional movement of the cell? If one looks at how bacteria respond chemotactically, it seems that attractants are detected by specific *chemoreceptors* at the cell surface.[145] For *E. coli,* receptors for at least five chemotactic chemicals (galactose, glucose, ribose, aspartate, and serine) have been identified.[145] Certain *E. coli* mutants lack various of these receptors but show normal responses towards the other chemicals;[145] other mutants fail to respond to any of the chemicals and are probably defective either in the transmission of information from the receptor to the cell's locomotory machinery or in the machinery itself.[145] Assuming that a cell has surface receptors for chemoattractants, a gradient may be detected either by a) a *temporal* mechanism, in which the cell samples the *milieu* at separate time intervals and compares the concentrations of the attractant, or b) a *spatial* mechanism, in which the cell compares the concentration of the attractant at two or more locations on its surface at the same time. For bacteria, the work of MacNab and Koshland [146] suggests that a temporal mechanism is involved. In these experiments, *Salmonella typhimurium* were subjected to sudden changes in the concentration of attractant; a sudden decrease in attractant concentration caused the bacteria to show incoordinated tumbling, whereas a sudden increase in concentration caused the bacteria to smoother, "supercoordinated," straight-line motion for several minutes. These effects would obviously result in a directional response up a gradient, and of course raise the interesting implication that bacteria would have some sort of "memory" to allow comparison of new and old information.

On the other hand, the available data suggest that a spatial mechanism is involved in leukocyte chemotaxis. Zigmond,[147] using time-lapse photography of leukocytes in slide-coverslip preparations, showed that stationary cells exposed to a chemotactic gradient made their first movement towards the chemotactic source. This indicates that a leukocyte can sense a difference in the number of chemotactic molecules across its own dimensions. However, we are still far from understanding how sensing of such a gradient leads to directional movement, i.e., how the actin-myosin [148-151] contractile elements of the cell are triggered and coordinated to produce the appropriate response. Nor do we know what happens to the specific chemoreceptors (if they exist) during cell movement, i.e., whether they remain randomly distributed over the cell surface or whether (as occurs for certain other surface components of neutrophils [152]) they slide to the tail of the cell.

It has been claimed that rates of locomotion are not affected during chemotaxis,[153,154] but this is contested by other workers,[138,155] who state that locomotion is stimulated in the presence of a chemotactic agent. Various other general effects of chemotactic agents have been reported. For instance, Goetzl and Austen [156] found that a two- to sixfold increase in the activity of the hexose monophosphate shunt was induced in human neutrophils; blocking of this shunt with 6-aminonicotinamide partially suppressed chemotactic responsiveness; [156] treatment with high doses of ascorbic acid, which stimulates the shunt, augmented both random migration and chemotaxis (as assessed in the Boyden system).[157] In addition, chemotactic agents have been reported to induce neutrophil lysosomal enzyme release,[158] cellular volume expansion,[158] calcium efflux,[159] and diminution of negative surface charge.[160]

It is likely that most treatments alleged to decrease chemotactic responsiveness in the Boyden system act by decreasing cellular locomotion. This could explain the chemotactic inhibition claimed to occur with cytochalasin B,[161] with treatments that raise intracellular levels of cyclic AMP,[162] and with various drugs (e.g., corticosteroids, chloroquine, phenylbutazone, ouabain).[163-165] Similarly, the chemotactic potentiation claimed for treatments that raise intracellular cyclic GMP [166] may be due to accelerated random locomotion. It has been reported that colchicine (and other drugs that affect microtubules) inhibit chemotaxis in the Boyden system.[167-169] Recently, however, it has been observed in slide-coverslip experiments that chemotactic polarization of neutrophils towards clumps of bacteria is unimpaired in the presence of colchicine (1×10^{-4} M).[152]

The studies of Ward and Becker [170-179] suggest that an essential step in the neutrophil's response to various chemotactic substances (e.g., $C\overline{567}$, C3 fragments, C5 fragments, and bacterial factors) involves the activation by the chemotactic agent of a cell-bound serine esterase (*activatable esterase*). This esterase appears to exist in an inert form (*proesterase 1*) and an active form (*esterase 1*). The latter is inhibited by organophosphorous inhibitors, such as diisopropyl phosphofluoridate (DFP) and a number of *p*-nitrophenyl ethyl phosphonate esters, thus inhibiting chemotactic responsiveness. The precise role of this esterase in the cell is unknown. A note of caution in the interpretation of these data was sounded by Woodin and his colleagues,[180,181] who questioned whether the effect of DFP and the *p*-nitrophenyl ethyl phosphonate esters is necessarily only due to their ability to phosphorylate. They suggested that, in the doses used, such chemicals may cause nonspecific inhibition of cellular locomotion, perhaps due to a detergent effect. Becker [176,182] has countered that such a suggestion conflicts with the finding that phenyl ethyl butylphosphonate and phenyl ethyl pentylphosphonate, analogs with poor phosphorylating activity, fail to inhibit chemotactic responsiveness. This controversy is not yet resolved.[124]

Does "negative chemotaxis" of leukocytes exist? Not with any certainty: various reports that leukocytes might be repelled by certain materials, e.g., kaolin,[133] have not been substantiated upon critical restudy.[183]

Finally, can chemotaxis be demonstrated *in vivo*? In rabbit ear chambers, accumulations of leukocytes have been observed at sites of thermal [184] or laser [124] burns. These experiments offer little information concerning whether true chemotaxis was involved, because no serious attempts were made to study the movement of individual cells. The leukocytes may have entered the injured area fortuitously during random movement and somehow became "trapped" there. However, Buckley [185] examined this point more precisely and found that heat injury in a minute area of an ear chamber was followed by active movement of neutrophils directly towards the damaged focus.

Phagocytosis

The most important task for neutrophils and mononuclear phagocytes in inflammatory sites is the ingestion and disposal of foreign or effete particulate material. This process of phagocytosis was, of course, recognized as a vital host defense mechanism by Metchnikoff.[186] For discussion, it can be subdivided into the following phases (see reviews by Elsbach [187] and Stossel [188]): a) attachment of particles to the cell surface (including contact and recognition), b) ingestion of particles by the cell (including formation of the phagosome and degranulation), and c) breakdown of particles within the cell (including microbicidal effects).

First, however, let us briefly consider some pertinent characteristics of neutrophils and mononuclear phagocytes. Neutrophils possess large numbers of cytoplasmic granules. These appear to be of two main types (Table 1): a) azurophil (or primary, i.e., appearing first in cellular development) granules which are large and electron dense, and contain various lysosomal hydrolases (e.g., acid phosphatase) and cationic proteins, as well as peroxidase (myeloperoxidase) and some lysozyme; and b) specific (or secondary, i.e., appearing later in development) granules which are smaller and less dense, and contain alkaline phosphatase, lysozyme, and lactoferrin but no lysosomal hydrolases and no peroxidase.[189,190] Neutrophil cytoplasm contains large quantities of glycogen; glycolysis rather than respiration is the major (>90%) mechanism for energy production in

Table 1—Major Granules of Rabbit Neutrophils—Their Contents

Azurophil (primary)—large, dense
Lysosomal enzymes
Peroxidase (myeloperoxidase)
Lysozyme (33%)
Cationic proteins
Specific (secondary)—smaller, less dense
Alkaline phosphatase
Lysozyme (67%)
Lactoferrin

the mature cell, thus permitting maintenance of effective function in hypoxic tissues and exudates.[189]

Mononuclear phagocytes are first identified as *promonocytes* in the bone marrow.[191] They enter the blood as *monocytes* and eventually become tissue *macrophages*, e.g., in connective tissue (where they are commonly called histiocytes), liver (Kupffer cells), lung (alveolar macrophages), lymphoid tissues (free and mixed macrophages) or serous cavities (pleural and peritoneal macrophages). This *bone marrow-blood-tissue* classification (Table 2) has been proposed as a working framework for the *mononuclear phagocyte system*,[192] to replace the time-honored but inappropriate term *reticulo-endothelial system* of Aschoff.[193] Monocytes and macrophages display abundant electron-lucent pinocytic lesicles.[191] Monocytes also contain azurophil granules, containing acid hydrolases and peroxidase.[191] Macrophages, particularly alveolar macrophages, show greater numbers of more heterogeneous dense granules; these contain large amounts of acid hydrolases and are probably secondary lysosomes resulting from the fusion of small Golgi-derived primary lysosomes with endocytic vacuoles.[191] Monocytes and macrophages obtain energy from respiration and glycolysis; [191] it has been noted that macrophages in different situations develop different metabolic properties, e.g., alveolar macrophages show greater rates of oxygen consumption than peritoneal macrophages.[191]

Attachment Phase of Phagocytosis

Little is known about the phagocyte's way of recognizing what it should attack. In some cases (e.g., the ingestion of polystyrene beads [194]), phagocytosis can proceed in the apparent absence of serum. In most circumstances, it is clear that coating, or *opsonization*, of particles by serum factors is of paramount importance in accelerating phagocytosis of, e.g., microorganisms. Opsonic serum factors that have been so far characterized belong to two main groups. First, there are heat-stable IgG_1 and IgG_3 specific antibodies directed against surface components of the par-

Table 2—The Mononuclear Phagocyte System

Bone marrow—promonocytes
Blood—monocytes
Tissues—macrophages
Connective tissue (histiocytes)
Liver (Kupffer cells)
Lung (alveolar macrophages)
Lymphoid tissues (free and fixed macrophages)
Serous cavities (pleural and peritoneal macrophages)

ticles; and secondly, there are heat-labile opsonic fragments of C3 which can become firmly bound to particles when the complement system is triggered.[188,195] Following contact of the cell with an opsonized particle, attachment presumably occurs by linkage to specific cell surface receptors for antibody (probably an Fc receptor) or for the C3 fragment.[188,195]

Ingestion Phase of Phagocytosis (Including the Formation of the Phagosome and Degranulation)

Engulfment occurs in the following sequence: the cell extends small cellular expansions or pseudopods which become closely applied to the surface of the attached particle; these processes pinch together, meet, and fuse around the particle; the resulting phagosome is drawn into the cell.[189] Using cinemicrophotography and electron microscopy, it has been demonstrated that cytoplasmic granules converge on the forming phagosome, fuse with it, and discharge their contents into the vacuolar lumen around the particle.[189] This process is called *degranulation*. In neutrophils, it appears that the specific granules fuse with the phagosome slightly before the azurophil granules;[196] this sequential fusion may relate to pH changes inside the vacuole.[197] As we shall see later (in the section on Mediators), granule contents can be released to the outside of the cell during phagocytosis,[198] presumably due to leakage from incompletely closed phagosomes.

Ingestion is an energy-requiring process [199] and is usually dependent upon the presence of divalent cations, Ca^{2+} and Mg^{2+}.[200] Inhibition of ingestion has been reported with drugs that increase intracellular levels of cyclic AMP,[188] but this has been disputed.[178] It is generally agreed that cytochalasin B inhibits phagocytosis,[201] but there is conflict about the effect of colchicine, some workers claiming inhibition,[188,202] whereas others have reported that it has no significant effect.[203] Engulfment by neutrophils is nonspecifically stimulated by the binding of a "leukophilic" γ-globulin;[204] this has been attributed to the action of "Tuftsin," a tetrapeptide fragment liberated from the γ-globulin molecule by an enzyme on the outer surface of the cell.[205] In addition, *activation* of phagocytic and microbicidal capacity occurs when mononuclear phagocytes enter inflammatory foci or when they are exposed to certain lymphocyte products [191] (perhaps MIF—see section on Mediators).

Once extensive endocytosis has occurred, the phagocyte is unable to ingest particles,[188] suggesting that phagocytic *receptor sites* may be lost from the surface because they are internalized within phagocytic vacuoles; mononuclear phagocytes (but not neutrophils) have the capacity with time to regain endocytic ability, probably because they can synthesize new surface receptors.[206] Interestingly, phagocytosis by neutrophils is not accompanied by internalization of membrane sites involved in the specific transport of amino acids.[207] However, such sites are internalized if the cells are treated with colchicine or vinblastine, suggesting (in line with other studies [152,208]) that microtubules may be somehow involved in controlling the movement of membrane components during phagocytosis.[209,210]

Processes Leading to the Breakdown on Particles Inside the Phagocytic Vacuole (Including Microbicidal Effects)

During phagocytosis (and after exposure to endotoxin or digitonin [211] or chemotactic factors [156]), neutrophils show a burst of metabolic activity, characterized by a two- to threefold increase in oxygen consumption, increased hydrogen peroxide (H_2O_2) formation, and an increase (to 10%, from a resting level of 1%) in the amount of glucose metabolized via the hexose monophosphate pathway (HMP).[212] There is significant dispute about the primary oxidase involved in this metabolic burst. One theory holds that an NADPH oxidase [213] catalyzes the oxidation of NADPH, forming H_2O_2 and $NADP^+$ which is required for operation of the HMP. Another theory is that the primary oxidase is NADH oxidase [212,214] which catalyses the oxidation of NADH, forming H_2O_2 and NAD^+. According to this latter view, the HMP is then stimulated by one or more of three possible pathways, the relative importance of each depending upon the species of origin of the cells. First, there may be transhydrogenation ($NAD^+ + NADPH \rightleftharpoons NADH + NADP^+$) to produce the $NADP^+$ required for the HMP, e.g., in human cells.[215] Secondly, a slight excess of pyruvate could be involved (with NADPH-linked lactate dehydrogenase) in oxidizing NADPH to $NADP^+$ at low pH, e.g., in guinea pig cells.[212,215] And thirdly, some of the H_2O_2 generated by the NADH oxidase may oxidize reduced glutathione (GSH) to yield oxidized glutathione (GSSG), which in turn oxidizes NADPH to $NADP^+$, e.g., in rat cells.[216] Recent ultrastructural cytochemical studies indicate that NADH oxidase is present on the neutrophil surface; [217] this enzyme would, therefore, be included within the phagosome membrane during internalization of ingested particles. H_2O_2 has also been detected cytochemically inside the phagocytic vacuole.[218]

Although lysosomal hydrolytic enzymes derived from the azurophil granules can degrade digestible material (including dead bacteria) within the phagocytic vacuole, they are unlikely to be a major cause of microbial death. Instead, antimicrobial activity has been attributed to an array of other factors (Table 3) (see reviews by Klebanoff [219,220]). These include: the acid pH inside the vacuole; [197] cationic proteins from azurophil granules

Table 3—Antimicrobial Agents Inside the Neutrophil Phagosome

Acid pH
Cationic proteins (e.g., phagocytin)
Lysozyme
Lactoferrin
Superoxide anion
Hydrogen peroxide
Hydrogen peroxide-myeloperoxidase-halide system

(e.g., "phagocytin" [189]); lysozyme (a lytic enzyme found in both types of granules); [189] lactoferrin (a bacteriostatic iron-binding protein from specific granules);[221] superoxide anion (O_2) derived from the reduction of oxygen; [222] and H_2O_2, particularly in association with myeloperoxidase and halide anions [219,220,223,224] or, somewhat less effectively, with ascorbic acid and traces of copper.[202,225]

H_2O_2 is a very important component in the microbicidal armamentarium of the phagocyte. Klebanoff clearly showed that myeloperoxidase and halide ions (I^-, Cl^-, or Br^-) greatly enhance the effectiveness of H_2O_2 against bacteria, fungi, and viruses.[219,220,223,224] The mechanisms involved in killing are uncertain, but they may include iodination (if I^- is utilized) or the formation of chloramines or toxic aldehydes.[224] The phagocyte apparently protects itself from the effects of free H_2O_2 by dealing with it in two ways: a small amount may be used up in the oxidation of reduced glutathione [216] but most is broken down in the cytoplasm by catalase.[212]

Lately, attention has focused on the potential antimicrobial role of superoxide anion.[226] This is a highly reactive radical formed by the one electron reduction of oxygen [222] and destroyed by superoxide dismutases.[227,228] Its production is stimulated by phagocytosis,[229] and it has been proposed as an intermediate in the formation of H_2O_2. The interesting suggestion that superoxide is bactericidial (perhaps via the formation of toxic hydroxyl radicals) [230] has not yet been clearly substantiated.

Defects of Leukocytic Function

In recent years, tremendous interest has been stirred by the realization that certain patients with recurrent infections may have functionally defective leukocytes. For instance, the neutrophils might not migrate normally, or they might show poor chemotactic responsiveness or phagocytic ability, or, worse yet, they might have a diminished microbicidal capacity. Not infrequently, more than one of these defects may be found in an individual patient. Table 4 sets out the major ways in which leukocytic function can be significantly disturbed and lists some clinical syndromes that seem to fit these disorders.[140,188,215,219,231-235]

Table 4—Defects of Leukocytic Function

Functional defect	Major clinical syndromes
Neutropenia	Leukemias Drug-induced agranulocytoses Cyclic neutropenia
Disorders of migration and chemotaxis	Intrinsic cellular dysfunctions Chediak-Higashi syndrome Lazy leukocyte syndrome Familial defect of chemotaxis Job's syndrome Neutrophil actin abnormality Diabetes mellitus Severe bacterial infection Inhibitors of locomotion Inhibitors in serum Drugs (e.g., corticosteroids) Deficiencies of chemotactic factors Complement deficiencies Chemotactic factor inactivator in serum
Disorders of phagocytosis (attachment, ingestion, degranulation)	Opsonin deficiencies Hypogammaglobulinemia Complement deficiencies (C3) Sickle cell disease Impaired engulfment Tuftsin deficiency Drugs (e.g., morphine analogs) Impaired degranulation Chediak-Higashi syndrome Drugs (e.g., corticosteroids, antimalarials)
Disorders of microbicidal mechanisms	Impaired H_2O_2 production Chronic granulomatous disease Glucose-6-phosphate dehydrogenase deficiency Drugs (e.g., hydrocortisone, sulfonamides) Myeloperoxidase deficiency

Neutropenia

Strictly speaking, this is a reduction in the number of cells rather than a functional defect, but it is included here for convenience. The most common causes are the leukemias or the drug-induced agranulocytoses. A rarer and more obscure condition is cyclic (periodic) neutropenia in which profoundly low neutrophil counts occur every 21 days, associated with the development of skin infections, otitis, and arthritis.[232]

Disorders of Migration and Chemotaxis

This group comprises the defects resulting in poor mobilization of cells to inflamed sites. It is likely that most of these are due to intrinsic or extrinsic factors that affect cellular locomotion, although it has been claimed that certain patients may show a selective defect in leukocytic sticking to endothelium (e.g., in trimethylaminuria [236]), or that the leukocytes may be specifically unable to recognize and respond appropriately to a chemotactic gradient.[140]

The major clinical syndromes in this group may be classified in terms of a) defects in the cells themselves (i.e., the intrinsic dysfunctions), b) defects due to the presence of inhibitors of locomotion, or c) defects resulting from a lack of chemotactic factors (Table 4).

Intrinsic Cellular Abnormalities of Leukocytic Locomotion. Included amongst these disorders are the following: the *Chediak-Higashi syndrome*,[237] an autosomal recessive disease characterized by the presence of giant azurophil (peroxidase-containing) granules in leukocytes and an increased susceptibility to infections; the *lazy leukocyte syndrome*,[238] characteristically showing defective random locomotion of leukocytes, neutropenia (perhaps because cellular hypomotility slows the egress of cells from the bone marrow), and repeated infections; an apparently *familial defect of chemotaxis* [159,239] in which susceptibility to infection was correlated with poor chemotactic responsiveness, despite a claim of normal random locomotion; *Job's syndrome*,[240] in which fair-skinned red-haired girls with high serum IgE levels [241] suffer recurrent "cold" staphylococcal abscesses; and a recently described condition in which repeated infections were attributed to a *neutrophil actin abnormality*.[242] In addition, the leukocytes from patients with *diabetes mellitus* have been reported to show poor chemotactic responsiveness; this defect could apparently be corrected by incubating the cells in medium containing insulin and glucose.[243] Diminished leukocytic locomotion has also been found in patients that were acutely ill with *severe bacterial infections*.[244] In each of these conditions, defective locomotion was detected by testing the cells in the Boyden chemotactic chamber: [136] fewer cells than usual crawled into or through the filter in response to a chemotactic target solution. Inhibition of responsiveness in this system has usually been interpreted as reflecting defective chemotaxis. In most cases, however, an impairment of random locomotion has not been rigorously excluded. It might be argued that this is a trivial point, because impaired locomotion will obviously cause impaired chemotaxis. However, in seeking to understand and perhaps ultimately correct the defects in these patients, it is axiomatic that the primary mechanism should be clearly delineated.

An exciting recent development has been the demonstration by Snyderman and Stahl [245] of defective monocyte chemotaxis (as assessed in the Boyden system) in patients with neoplastic disease, the Wiskott-Aldrich syndrome, or chronic mucocutaneous candidiasis. In many of the patients with tumors, immunotherapy with BCG or removal of the tumor restored responsiveness of the monocytes.

Inhibitors of Leukocytic Locomotion. These inhibitors have been found in the serum of certain patients. Such inhibitors also diminish the *in vitro* locomotion of cells from normal individuals. This effect has been detected with serum from patients with rheumatoid arthritis [246] (possibly due to a cell-directed effect of rheumatoid factor complexes),[246] or from patients with raised serum IgE levels [247,248] (possibly due to a histamine-mediated accumulation of cyclic AMP in the leukocytes).[169,249] Two of the reported cases in this group, however, presented only with a history of recurrent infections; [250,251] in the first, it was claimed that the serum caused a defect in chemotactic responsiveness,[250] but more critical study of the other case (by measuring lengths of photographically recorded migratory tracks of cells observed in the slide-coverslip system of Harris) [134] clearly revealed a marked depression of random locomotion.[251] It has also been claimed that certain drugs in clinical use (e.g., corticosteroids, quinoline derivatives, phenylbutazone) may impair leukocytic movement.[165]

Deficiencies of Chemotactic Factors. These may occur in patients with genetic deficiencies or abnormalities of complement factors, especially the chemotactic factor substrate C3 [252,253] and C5 [254] (see section on Mediators). The significance of these deficiencies with respect to leukocytic chemotaxis is not clear. Such patients may show an increased susceptibility to infection, but this can probably be attributed to a defect in the opsonizing capacity of the serum (see next section) rather than a lack of chemotactic agents. Bacterial proteases may liberate chemotactic fragments from normal C3 and C5 [255] (see section on Mediators), but it is most likely that leukocytes are attracted to sites of infection by exogenous chemotactic factors released from the bacteria themselves.

An interesting recent development has been the identification, in serum, of *chemotactic factor inactivators* (CFI) which inactivate the chemotactic fragments of C3 and of C5, the $\overline{C567}$ complex, and a bacterial chemotactic factor.[140,165,256] Small amounts of CFI are detectable in normal human serum; strikingly elevated levels have been reported in patients with Hodgkin's disease [140,165,257] or Bruton-type agammaglobulinemia.[165] In contrast, deficient CFI levels have been found in patients with both pulmonary emphysema and α_1-antitrypsin deficiency,[258] a finding that may be relevant to the pathogenesis of emphysema (as discussed in the section on Mediators).

Disorders of Phagocytosis (Attachment, Ingestion, and Degranulation)

Defective Attachment. Defective attachment and uptake of microorganisms is usually explained by poor opsonization. This may be due to a lack of antibodies (e.g., in hypogammaglobulinemia) or an abnormality in the complement system (especially in certain patients with C3 deficiency).[252,253] In sickle cell disease, it appears that susceptibility to pneumococcal septicemia and meningitis is due to a specific lack of opsonin for pneumococci.[259]

Diminished Ingestion. This has also been attributed to a deficiency of Tuftsin, said to occur either as a familial defect or following splenectomy.[260-262] In addition, certain drugs (e.g., morphine analogs)[263] may cause impaired ingestion.

Impaired Degranulation. In the neutrophils and monocytes of patients with the Chediak-Higashi syndrome, the giant cytoplasmic granules fail to fuse normally with phagosomes, resulting in defective degranulation and decreased discharge of granule contents into phagocytic vacuoles.[264] The process of degranulation may also be affected by drugs, e.g., colchicine,[188,202] corticosteroids, antimalarial drugs.[265] Another, most interesting failure of granule–phagosome fusion occurs when macrophages ingest viable *Mycobacterium tuberculosis*[266] or *Toxoplasma gondii*[267] organisms; this phenomenon, which favors parasitic survival in normal hosts is now explained (see Addendum).

Disorders of Microbicidal Mechanisms

The key role of the leukocyte H_2O_2–myeloperoxidase–halide system in microbial killing is emphasized when we examine the clinical syndromes associated with either impaired H_2O_2 production or myeloperoxidase deficiency (see reviews).[215,219,232-235] The most important and dramatic example of impaired H_2O_2 production is *chronic granulomatous disease* (CGD). This was first recognized as a distinct clinical entity in 1957[268,269] and was identified as being due to a leukocytic microbicidal defect in 1966.[270,271] CGD is an inherited disease, typically affecting males; it commonly causes death before 7 years of age. Children with this condition usually present with multiple recurrent granulomatous infections of the skin and lymph nodes and often develop pneumonia, liver abscesses, and osteomyelitis.[234] There appears to be no defect in uptake of organisms by the leukocytes.[234] For degranulation, however, the picture is less clear: an abnormality has been claimed by some workers[271] and refuted by others;[272,273] recent work suggests that any impairment of degranulation (as measured by the external discharge of lysosomal enzymes) occurs only in the early stages following phagocytosis and that no differences from normal are found at 30 minutes.[274]

It appears that the major defect in CGD lies in an inability of leukocytes to respond with the burst of metabolic activity that usually accompanies phagocytosis (see reviews),[215,219,232-235] perhaps due to a lack of NADH oxidase [215,234] (although the identity of the deficient enzyme is disputed).[270] This results in a failure of the cells to make H_2O_2 and hence leads to failure of the H_2O_2–myeloperoxidase–halide system; in addition, neutrophil superoxide production is deficient in CGD.[230,275] As to the microorganisms which most commonly produce serious infections in these patients, catalase-producing bacteria (e.g., *Staphylococcus aureus*) are high on the list, whereas H_2O_2-producing, catalase-negative bacteria (e.g., pneumococci) do not cause significant problems.[234] The catalase-negative bacteria unwittingly contribute to their own demise by providing sufficient H_2O_2 for the H_2O_2–myeloperoxidase–halide system to function effectively inside the phagocytic vacuole. On the other hand, the catalase produced by catalase-positive bacteria destroys the small amounts of H_2O_2 that the bacteria may produce, thus preventing killing. This concept has been elegantly exploited by Johnston and Baehner [276] who showed that improved killing of *Staphylococcus aureus* and *Serratia marcescens* could be induced in CGD neutrophils if the cells were also allowed to phagocytize latex spherules coated with glucose oxidase (which acts as an H_2O_2 generator by reacting with intracellular glucose).[277]

An important diagnostic procedure for CGD is the nitro blue tetrazolium (NBT) test. When yellow oxidized NBT is added to phagocytizing neutrophils, the dye enters the phagocytic vacuoles and is reduced to a dark blue formazan; the mechanism of this NBT reduction has not been identified (see review by Segal).[278] Baehner and Nathan [279] discovered that CGD neutrophils show defective reduction of NBT during phagocytosis.

Another potential cause of defective H_2O_2 production in cells is glucose-6-phosphate dehydrogenase (G6PD) deficiency. Patients with this deficiency show abnormal bactericidal activity and increased susceptibility to infection, but only if the G6PD level is near zero.[280]

Exposure of leukocytes to hydrocortisone inhibits oxygen consumption, H_2O_2 production, and NADH oxidase activity and causes diminished intracellular microbicidal capacity.[281] This may be a factor in the predisposition to infection during prolonged corticosteroid therapy. Certain sulfonamides have also been reported to interfere *in vitro* with leukocytic H_2O_2-dependent microbicidal function.[282]

From what we have said about the importance of the H_2O_2-myeloperoxidase-halide system, leukocytes with a myeloperoxidase deficiency might be expected to show a microbicidal defect. Although such a defect has been found in association with recurrent infections,[238] most individuals with myeloperoxidase deficiency suffer no ill-effects, suggesting that the lack of myeloperoxidase may allow the intracellular accumulation of increased amounts of directly microbicidal H_2O_2.[220,235]

Before leaving the disorders of leukocytic function, let us briefly consider a special example. Newborn infants, especially if premature, have an increased susceptibility to infection.[219] This has been attributed primarily to inefficient opsonizing capacity, possibly due, in part, to deficiencies in serum complement components (e.g., C3 [284]); no intrinsic leukocytic microbicidal defect has been convincingly demonstrated.[233]

Systemic Effects

Patients with inflammatory disease show various systemic signs of their illness. The best studied are *fever* and *leukocytosis*. Another well-known change is the *increased erythrocyte sedimentation rate*, attributed mainly to an alteration in plasma protein composition that somehow leads to enhanced erythrocyte rouleaux formation.[285,286] In addition, the *concentration of iron in the plasma is commonly decreased*, possibly because of the release of neutrophil lysosomal apolactoferrin; this removes the iron from plasma transferrin to form Fe-lactoferrin, which is then selectively taken up by blood monocytes and by macrophages of the liver, spleen, and lungs.[287] Because iron is required for microbial growth, it has been proposed that low plasma iron levels confer a host advantage in dealing with infection.[288]

Fever

Fever, an abnormally high body temperature, is a characteristic clinical sign of inflammatory disease, whether due to infections, tissue necrosis, or hypersensitivity reactions.

Normal body temperature is maintained by an appropriate balance of heat loss (enhanced by skin vasodilatation and sweating) versus heat conservation and generation (enhanced respectively by skin vasoconstriction and shivering). This balance is controlled by a hypothalamic thermoregulatory center which senses temperature changes in the perfusing blood. Thus, when the hypothalamus senses a fall in the control temperature, autonomic triggering induces peripheral vasoconstriction. This produces, in turn, a fall in skin temperature, sensory input from skin thermoreceptors, and reflex stimulation of somatic motor nerves to skeletal muscles. This results in heat production due to shivering.

A coherent view of the pathogenesis of fever has emerged over the past decade or so, largely from the studies of Wood and Atkins and their colleagues (see reviews).[289-292] It appears that fever can usually be attributed to the release of factors called *endogenous pyrogens* from host cells into the blood. These factors then exert their effects by a central action on the hypothalamus, rather like that described above for the effect of cold, i.e., resulting in skin vasoconstriction, shivering ("chills"), and hence an inappropriate elevation of the central temperature. The mode of action of endogenous pyrogens in the hypothalamus is not known, although there are indications [293] that prostaglandins may act as local neurohumoral transmitters; prostaglandin synthesis is inhibited by aspirin, indomethacin, etc., thus providing a plausible explanation for the antipyretic effects of such drugs (see section on Mediators). Endogenous pyrogens were first thought to be released only from neutrophils,[294,295] but subsequent studies have clearly shown that they can also be derived from blood monocytes,[296] peritoneal macrophages,[297] lung macrophages,[298] eosinophils,[299] and phagocytic cells of the liver (Kupffer cells),[300] spleen,[298] and lymph nodes.[298] Indeed, it has been demonstrated that monocytes and peritoneal macrophages produce larger amounts of pyrogen than do neutrophils.[296,297] Endogenous pyrogens appear to be proteins;[292,301,302] recent work shows that the pyrogen from human monocytes is larger (molecular weight, 38,000) and more acidic than the pyrogen from human neutrophils (molecular weight, 15,000).[303]

What causes release of endogenous pyrogens? Pyrogen release from leukocytes is triggered following exposure of the cells to various stimuli [292] (Table 5): a) phagocytosis, b) bacterial endotoxins, c) antigen–antibody complexes (requiring complement for pyrogenicity if in antigen excess),[304,305] d) certain viruses, e) a pyrogenic *lymphokine* (a product released from sensitized lymphocytes treated with specific antigen—see section on Mediators),[306] or f) certain steroids (e.g., etiocholanolone—a metabolite of androgens) and bile acids.[307]

It was claimed that neutrophil pyrogen is a lysosomal cationic protein,[308] but this has been disputed;[292,309] other investigators have shown that very little preformed, active pyrogen is present in unstimulated cells [292]—the little that can be detected has been localized in the supernatant fraction rather than in the granules.[309] Following appropriate stimulation *in vitro*, human blood neutrophils release detectable amounts of pyrogen into the medium after 2 hours, and this continues for about 12 hours.[292] Release is inhibited if the cells are treated in the first 2 hours with inhibitors of RNA synthesis, or glycolysis; such inhibitors have no effect if added 2 hours after cell stimulation.[292] Cyanide, an inhibitor of oxidative

Table 5—Fever—Factors Provoking Release of Endogenous Pyrogen From Leukocytes

Phagocytosis
Endotoxins
Antigen-antibody complexes
Certain viruses
Pyrogenic lymphokine
Certain steroids (e.g., etiocholanolone)

metabolism, does not prevent release at any stage.[292] On the other hand, no pyrogen is formed if the cells are disrupted, or if they are kept at 0 C.[292] More pyrogen appears when the cells are suspended in larger volumes or are placed in fresh medium, indicating that some sort of feedback inhibition mechanism may regulate release.[292] Interestingly, corticosteroids and estrogens suppress endogenous pyrogen release *in vitro*, perhaps explaining at least part of their antipyretic effect *in vivo*. [307]

In summary: Fever can generally be explained by the action of endogenous pyrogens on the hypothalamus. These factors are released into the blood from intact neutrophils and mononuclear phagocytes; release occurs following phagocytosis or exposure to endotoxins or antigen–antibody complexes. It is reasonable to suppose that such processes are involved in clinical inflammatory states, although why the body should choose to respond in this manner is not yet clear. In isolated circumstances, elevation of body temperature appears to have a favorable effect in combating infection, e.g., in syphilis or gonococcal urethritis;[310] on the other hand, fever vastly enhances host susceptibility to the lethal action of bacterial endotoxins.[310]

Leukocytosis

Patients with acute inflammatory disease, particularly due to bacterial infection, show elevated levels of neutrophils in the blood that are presumably geared to providing an abundant supply of phagocytic cells to maintain the body's defenses. As well as showing the well-known "shift to the left" (characterized by the presence of circulating band forms, metamyelocytes and sometimes myelocytes), the individual neutrophils in such patients may also display distinctive cytoplasmic features (when examined with Wright's stain), e.g., Dõhle bodies [311] (blue amorphous inclusions, identified as aggregates of rough endoplasmic reticulum),[312] "toxic" granules (unusually large azurophil granules),[312] and vacuolization.

Before discussing mechanisms that may be involved in leukocytosis, let us briefly consider how neutrophils and their precursors are normally distributed in the body. Proliferation of primitive precursor cells in the bone marrow gives rise to a progressively expanding "mitotic pool" consisting of myeloblasts, promyelocytes, and myelocytes. After cell division ceases, cellular maturation proceeds in the bone marrow's huge "neutrophil reserve" (normally containing more than ten times the total number of neutrophils present in the blood). Kinetic studies show that just over half of the blood neutrophils are usually in a "marginated pool," stuck to the walls of small blood vessels, particularly in the lungs. The mean half-disappearance-time from the circulation is about 6 hours for neutrophils, and their turnover rate (reflecting death in the blood or loss to the tissues) is of the order of 10^{11} cells/day.[313]

Factors thought to be involved in regulating the number of circulating neutrophils (see review by Golde and Cline)[314] are listed in Table 6. Exercise or epinephrine induce release of leukocytes from the marginated pool, producing a rapid rise in mature neutrophils in the circulating blood.[313] In contrast, large numbers of less mature cells appear transiently in the circulation within a few hours of injecting various *neutrophil-releasing factors.* This phenomenon, which apparently results from the mobilization of preformed cells from the bone marrow neutrophil reserve,[313] occurs in response to the injection of *plasma* from a) rats subjected to repeated peritoneal lavage (washing out large numbers of neutrophils),[315] b) dogs rendered leukopenic by treatment with vinblastine sulfate or nitrogen mustard,[316] or c) animals injected a few hours previously with endotoxin or bacterial antigens.[315,317] Gordon and his colleagues [315] suggested that the active principle (*leukocytosis-inducing factor*—LIF) present in their experiments caused increased blood flow through the bone marrow, thus leading to enhanced leukocytic release. However, the modes of action, as well as the nature and origin of the principle(s) involved remain uncertain. An interesting development is the finding of *leukocyte-mobilizing factor* activity in a C3 fragment cleaved from the third component of serum complement by the action of C$\overline{42}$ enzyme.[318] This may explain why certain patients with C3 deficiency fail to show a normal leukocytosis in response to infection.[253]

Table 6—Leukocytosis—Factors Influencing Number of Circulating Neutrophils

Factors	Major effects on neutrophils
Exercise, epinephrine	Release from marginated pool, e.g., in lung
Neutrophil-releasing factors, e.g., leukocytosis-inducing factor, C3 fragment	Release from neutrophil reserve e.g., in bone marrow
Colony-stimulating factor (CSF)	Stimulation of proliferation of colony-forming cells in bone marrow
Granulocytic chalone	Inhibition of proliferation of neutrophil precursors in bone marrow

Stimulation of granulopoiesis in the bone marrow occurs in response to *colony-stimulating factor* (CSF).[319] This is so named because it specifically stimulates proliferation of committed myeloid stem cells, the *colony-forming cells*; these include the precursors of granulocytes and monocytes. When bone marrow cells are suspended in tissue culture medium made semisolid by the inclusion of agar or methyl cellulose, proliferation does not occur unless CSF is incorporated into the cultures; [319] culture of the cells in the absence of CSF for 24 hours leads to death or loss of proliferative capacity.[319] CSF is a glycoprotein found in serum and urine.[319,320] It can be extracted from salivary glands, lung, thymus, and various other tissues; [320,321] the cells responsible for CSF generation *in vivo* have not been identified, although macrophages, fibroblasts, or endothelial cells have been suggested as possible sources, but on no firm basis.[320,321] Colony-stimulating factor is also released from many cell lines in culture medium; [320] it has also been found that CSF is released by lymphocytes following stimulation with antigen or mitogens.[322-324] When injected into animals, partially purified CSF does not provoke neutrophil release from the bone marrow reserve but does stimulate an increased production of new granulocytes and monocytes in the bone marrow, with a consequent slow rise of these cells in the blood, maximal at 24 to 48 hours.[319] It is intriguing that endotoxin and other bacterial products induce an enormous rise in serum CSF levels within minutes of injection.[320,321] Indeed, it has been proposed that bacterial products may play a major role in determining CSF production in the body and hence granulopoiesis.[320]

Another factor that may contribute to granulopoietic regulation is *granulocytic chalone*.[325] This is a substance, probably a polypeptide,[326] present in immature and mature cells of the granulocyte series; it is claimed to specifically inhibit the proliferation of myeloid precursor cells in the bone marrow.[325,326]

In summary: The number of circulating neutrophils can be affected by a) factors causing release of mature cells from the marginated pool in the tissues (e.g. exercise, epinephrine), b) factors causing release of immature cells from the neutrophil reserve in the bone marrow (neutrophil-releasing factors, e.g., LIF, C3 fragment), or c) factors affecting granulopoiesis in the bone marrow (e.g., colony-stimulating factor, which induces proliferation of granulocyte precursors, versus a granulocytic chalone, which induces inhibition). Although the mechanisms have not been clearly defined, it is likely that the neutrophil-releasing factors and colony-stimulating factor play significant roles in the systemic leukocytosis of inflammatory disease.

Mediators

Many of the processes involved in inflammation are attributed to chemical agents called *mediators*. Some bacterial products can cause vascular leakage [39] or attraction of leukocytes.[42,135] These are included among the so-called exogenous mediators and certainly play some part in the responses to bacterial infection. However, the so-called endogenous mediators (that is, those derived from the injured host) are of more general interest and importance. As shown in Table 7, these can be classified into two major groups (see review by Ryan [327]): those from *plasma* and those from *tissues*.

Factors Released From Plasma

In plasma, there are three interrelated mediator-producing systems (Table 7): a) the kinin system, b) the complement system, and c) the clotting system.

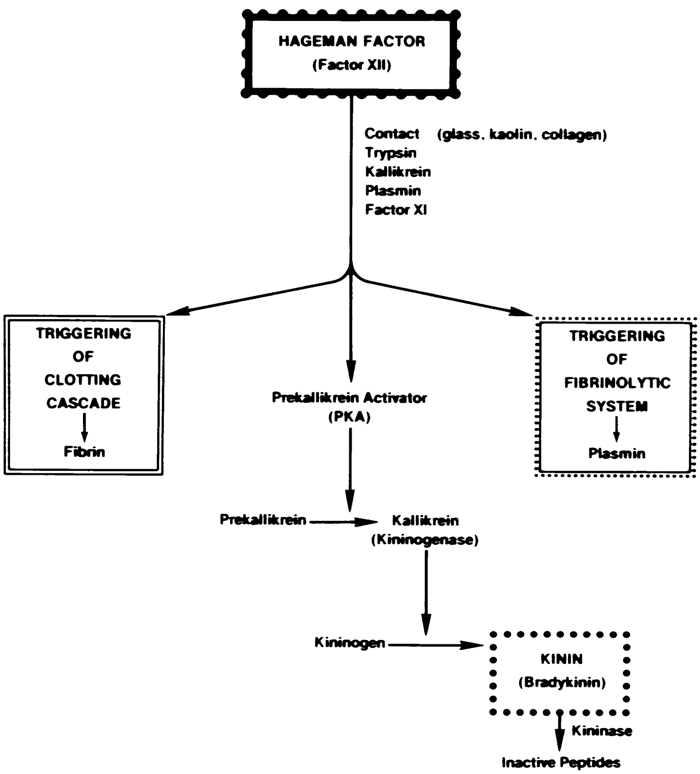
Table 7—Classification of Endogenous Mediators of Inflammation

Origin	Major groups	Major mediators
Plasma	Kinin system	Bradykinin
		Kallikrein
		Plasminogen activator
	Complement system	C3 fragments
		C5 fragments
		$C\overline{567}$ complex
		C kinin
	Clotting system	Fibrinopeptides
		Fibrin degradation products
Tissues	Vasoactive amines	Histamine
		5-Hydroxytryptamine
	Acidic lipids	Slow-reacting substance of anaphylaxis
		Prostaglandins
	Lysosomal components	Cationic proteins
		Acid proteases
		Neutral proteases
	Lymphocyte products	Migration inhibitory factor
		Chemotactic factors
		Lymphotoxin
		Skin reactive factors
		Mitogenic factor
		Lymph node permeability factor
	Others	Endogenous pyrogens
		Leukocytosis factors
		Substance P
		Neurotensin
		Cyclic AMP

Kinin System

In 1954, Armstrong *et al.*[328] discovered that exposure of plasma to glass led to the appearance of a substance which caused pain and stimulated smooth muscle contraction. A year later, Miles and Wilhelm [329] described the development of a permeability-inducing factor when serum was diluted. This was called *PF/dil*, but it was soon found that its development depended not upon the dilution itself (which simply diluted an inhibitor of the PF) but upon contact of the serum with the glass tubes in which the dilution was performed (see review by Miles [330]). Margolis [331] then established that glass activation depended upon triggering of Hageman factor (Factor XII of the clotting system). It is now recognized that this leads to the production of pharmacologically active *kinins*.[330,332]

Largely from the work of Cochrane and Wuepper [333-341] and Kaplan and Austen,[342-345] in which efforts have been made to isolate, purify, and characterize the individual components in the sequence, the scheme of the kinin system shown in Text-figure 2 has evolved. Hageman factor is



TEXT-FIGURE 2—The Plasma Kinin System.

activated by contact with substances having negatively charged surfaces, such as glass or kaolin, and with a variety of biologic materials, including collagen, basement membrane, sodium urate crystals, and cartilage.[339-344] In addition, activation occurs when Hageman factor interacts with trypsin, kallikrein (a later component of the kinin system), plasmin (the fibrinolytic enzyme), Clotting Factor XI, or bacterial lipopolysaccharides (endotoxins).[339-342,346] A claim that antigen–antibody complexes could directly activate Hageman factor [347,348] has been refuted.[349]

Activated Hageman factor has three effects: a) triggering of the clotting cascade (by activating Factor XI); b) triggering of the *fibrinolytic system* (by activating plasminogen proactivator to give plasminogen activator, which converts plasminogen to plasmin [350]); and c) *prekallikrein activator* (PKA) activity.[339,340,344,351] PKA, apparently a fragment of Hageman factor,[338] activates prekallikrein to form kallikrein.[337] Kallikrein acts as a kininogenase, i.e., it cleaves kininogen to produce kinin.[352] Upon such activation of the kinin system, the kinin produced is *bradykinin* (a nonapeptide).[330] Various secretions (e.g., saliva, pancreatic juice, sweat, tears), feces, and urine contain *tissue kallikreins* which cleave kininogen to produce a decapeptide kinin (called *kallidin*) that is then rapidly converted to bradykinin by a plasma aminopeptidase.[352]

Where does PF/dil (sometimes called *globulin PF*) fit into the scheme of the kinin system (Text-figure 2)? At one stage, it was thought to be responsible for activating prekallikrein.[330,352] However, more recent work shows that PF/dil is different from PKA,[334,335,338] and so its position in the kinin system is now uncertain. It may actually represent a functional combination of several factors (probably forms of activated Hageman factor [332]) rather than a specific molecular species.

Kinins are rapidly broken down by *kininases*, which are peptidases present in plasma and tissues.[352] Virtually complete inactivation of bradykinin occurs during a single passage through the lung circulation.[353] The kinin system is also held under control by the action of *kallikrein antagonists* in plasma and tissues; [352] one of these, from bovine tissues, is marketed under the name Trasylol. In addition (as we shall discuss later, in connection with hereditary angioneurotic edema), *C1 esterase inhibitor* not only acts against C1 esterase of the complement system, but also inhibits the kinin system by inhibiting the effects of activated Hageman factor and of kallikrein and plasmin.[351,354,355]

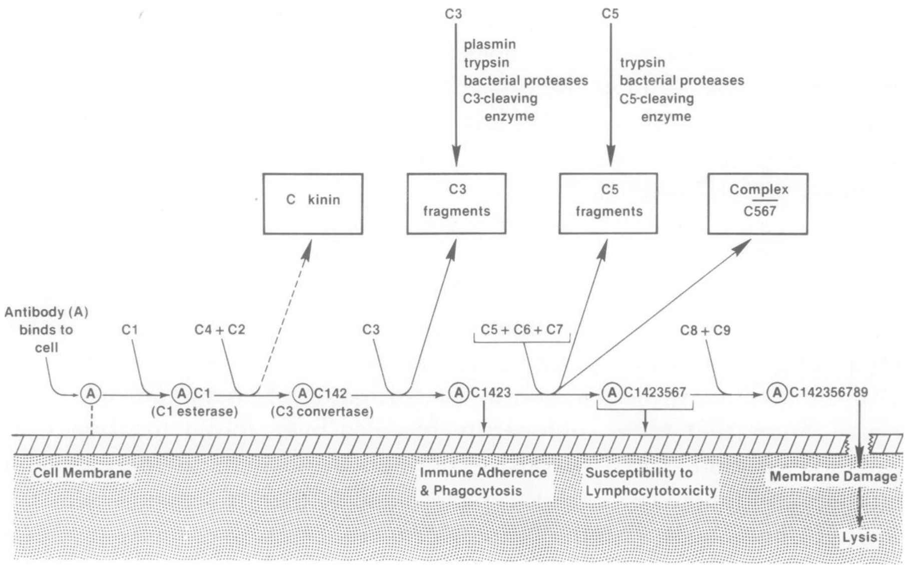
Bradykinin is the major effector agent of the kinin system. In very low doses it causes a) slow contraction of certain kinds of smooth muscle *in vitro*, b) dilatation of systemic blood vessels *in vivo*, thus inducing hypotension, c) pain when applied to the base of a blister or when injected into the skin, and d) increased vascular permeability following local injection (see review by Wilhelm [352]). It does not attract leukocytes in the Boyden chemotaxis system.[356] On the other hand, it has been claimed that kallikrein and plasminogen activator have chemotactic activity for neutrophils and mononuclear phagocytes; [357-359] kallikrein is thought also to be chemotactic for basophils.[360]

Complement System

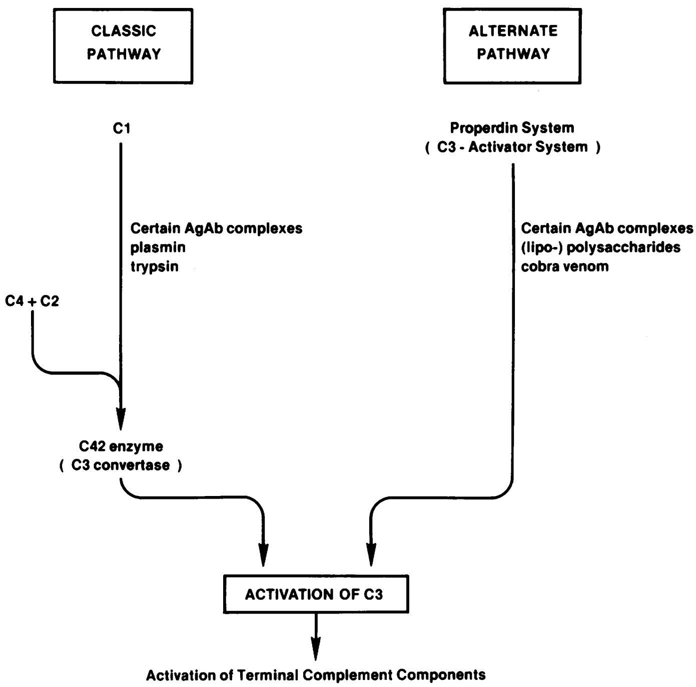
When injected into the circulation of an animal, serum that has previously been incubated with immune precipitates causes a severe reaction resembling anaplylactic shock. This was described by Friedberger [361] in 1910; he attributed the reaction to the formation of *anaphylatoxin* in the serum. Since that time, such serum has also been found to cause contraction of smooth muscle *in vitro*,[362] to induce a local increase in vascular permeability if injected into the skin,[362] and to attract leukocytes in the Boyden chemotaxis system.[136] Such properties are due to by-products generated by the interaction of antigen–antibody complexes with the complement system.

The details of the cascade of steps involved in the classic complement reaction derive mostly from studies of lysis of sheep erythrocytes exposed to rabbit antibody (see reviews [351,363-366]). As outlined in Text-figure 3, C1 (consisting of three subunits: C1q, C1r, and C1s) is activated by the immune complex to form C1 esterase, which in turn acts upon C4 and then C2, leading to the formation of $C\overline{42}$ enzyme (called *C3 convertase*). C3 convertase cleaves C3 into C3a fragments (which are released into the medium—see later) and C3b fragments which can bind to the cell, forming $C\overline{423}$ enzyme; the binding of opsonic C3 fragments to the surface at this stage facilitates attachment (*immune adherence*) to phagocytic cells.[195] $C\overline{423}$ enzyme interacts with C5 (cleaving off C5a fragments—see later), followed by C6 and C7 (apparently with the production of $C\overline{567}$ complex); the cell at this stage shows an increased susceptibility to damage by lymphocytes.[367] Finally, there is binding of C8 and C9, leading to membrane damage and lysis of the cell.

After years of controversy, the *properdin system* described by Pillemer and associates [368,369] in 1954 has become established as an *alternate* pathway to activation of the complement system (Text-figure 4), i.e., bypassing (and leaving intact) C1, C4, and C2, but nevertheless causing activation of C3 and, hence, the later complement components (see reviews [351,366,370]). It appears that the recently described *C3 activator system* [371] is identical to the properdin system.[370,372] A series of serum components (e.g., properdin, Factor B, Factor D) have been identified as



TEXT-FIGURE 3—The Complement System and Biologically Active By-Products.



TEXT-FIGURE 4—Complement Activation.

being involved in the system, but all of the activation steps have not yet been precisely delineated.[366,370]

The classic pathway (involving C1, C4, and C2 with the production of C3 convertase) is activated by antigen–antibody complexes (as well as by nonimmunologic agents such as plasmin and trypsin [373]). The alternate pathway is activated by certain kinds of antigen–antibody complexes, various polysaccharides, bacterial lipopolysaccharides (endotoxins), and cobra venom [351,370] (Text-figure 4).

The complement system is held under control by the inherent instability of certain of its component enzymes and by the presence in serum of various inhibitors or inactivators, e.g., C1 esterase inhibitor (see later), and C3b inactivator (which destroys the hemolytic, immune adherence, and phagocytosis-promoting properties of surface-bound C3 fragments, and also acts to inhibit the alternate pathway.[366,374]).

In the present context, the significant role of the complement system is in the formation of biologically active by-products which can act as inflammatory mediators. As indicated in Text-figure 3, such by-products are produced as a result of the sequential activation of the complement system (by either the classic or alternate pathways) or by the direct action of various "extracomplementary" enzymes on either C3 or C5. These by-products include: a) *C3 fragments*—low-molecular-weight factors released during complement activation or from cleavage of C3 by plasmin,[375] trypsin,[376] bacterial proteases,[255] or C3-cleaving enzymes found in various tissues; [376-378] b) *C5 fragments*—low-molecular-weight factors released during complement activation or from cleavage of C5 by trypsin,[376] bacterial proteases,[255] or C5-cleaving enzymes found in lysosomes of neutrophils,[379] platelets,[380] and possibly other cell types; [381] c) *complex* $\overline{C567}$—a high-molecular-weight complex of C5, C6, and C7, resulting only from the sequential activation of the complement system (classic or alternate); and perhaps d) *C kinin*—a kinin-like peptide (possibly a split product of C2 [382]) isolated from the plasma of patients with hereditary angioneurotic edema.[383]

The major inflammatory effects of the complement system by-products are as follows (Table 8):

Increased Vascular Permeability. This has been attributed to the formation of anaphylatoxins, which act primarily as histamine-releasing agents (see later), although in some circumstances (e.g., in contracting smooth muscle) they may also be able to act independently of histamine release.[362] Anaphylatoxin activity has been described in both C3a fragments and C5a fragments,[362,384-390] although it is likely that the classic anaphylatoxin arising in activated guinea pig serum is predominantly

Table 8—Major Inflammatory Effects of Complement System By-Products

Complement by-product	Inflammatory effects: Vascular leakage	Chemotaxis of neutrophils	Others
C3 fragments	+	+	Leukocytosis
C5 fragments	+	+	Neutrophil (lysosomal enzyme release)
$C\overline{567}$ complex	−	+	Lysis of bystander cells
C kinin	+	−	−

C5a.[384] In earlier studies, it was difficult to demonstrate anaphylatoxin formation in whole human serum because of the presence of an anaphylatoxin inactivator,[391] but this was overcome by removal or inhibition of the inactivator.[390] Following injection into human skin, these fragments induce erythema and vascular leakage; [390,392] in such studies, C5a shows almost 1000 times the activity of C3a.[390]

Chemotactic Attraction of Leukocytes. In 1961, Hurley and Spector [112] demonstrated a rapid and massive infiltration of neutrophils into the dermis of rats injected with serum which has been previously incubated with minced tissue. They attributed this to an active principle derived from the action of a tissue factor on a heat-labile substrate in serum. One year later, Boyden [136] published his filter membrane method for assessing the chemotactic activity of substances in solution. As mentioned earlier (see section on Chemotaxis), Boyden found that a chemotactic factor was produced when unheated serum was incubated with antigen–antibody precipitates. Hurley [114] then showed that serum which had been incubated with minced tissue was also chemotactic in the Boyden system, and it was subsequently demonstrated (by direct observation and photographic recording of cell locomotion) that neutrophils were attracted towards tissue fragments which had been incubated in serum.[115]

Since these early experiments, it has become clear that tissue fragments and immune precipitates act upon the serum complement system to produce chemotactic agents (see reviews by Ward [140,376]). From studies using the Boyden system, Ward and his co-workers [356] initially proposed that the major chemotactic factor produced by the interaction of serum with immune precipitates is composed of the high-molecular-weight complex $C\overline{567}$. This has been contested by other groups. First, Stecher and Sorkin [393] reported that chemotactic activity could develop in C6-deficient serum incubated with immune precipitates. Secondly, Snyderman *et al.*[394,395] found that the major chemotactic factor formed in serum treated with preformed immune complexes or with endotoxin is of low molecular weight and has the characteristics of fragments of C5; they could detect no chemotactic activity that could be attributed to the complex $C\overline{567}$. The explanation for these conflicting results is not clear. It may reside in differences in technique, such as the use of filters with different pore sizes and different methods of scoring results. Alternatively, the results of Stecher and Sorkin might simply be due to a mechanism whereby a multicomponent system can compensate for an isolated deficiency by switching to the production of another active by-product. However, the findings of Synderman's group indeed suggest that a significant proportion of the activity first attributed to $C\overline{567}$ may be due to the presence or generation of active C5 fragments. This is not to deny that $C\overline{567}$ is an entity that might have chemotactic activity under certain circumstances (as confirmed by another group,[396]), as well as other effects (such as participation in *reactive lysis*, i.e., the lysis of unsensitized "innocent bystander" cells due to binding of the complex to the cell surface, followed by binding of C8 and C9 which results in membrane damage [397]).

The chemotactic factors formed when damaged tissue interacts with serum are probably derived from both C3 and C5. As already mentioned, C3- and C5-cleaving enzymes are present in various cell types; [376,377,379-381] the resulting fragments are chemotactic as well as anaphylatoxic.[376] The biologic significance of these complement-derived factors has been underscored by the finding of chemotactic C3 fragments in recently infarcted heart muscle [398] and in synovial fluids of inflammatory nonrheumatoid joint disease,[399] and by the finding of chemotactic C5 fragments in synovial fluids of rheumatoid arthritis [399] and extracts of tissue showing immunologic vasculitis [400] (see reviews by Ward [376,401]).

So far, we have considered the chemotactic attraction of neutrophils. Experiments using the Boyden system indicate that complement by-products also attract other kinds of leukocytes. Normal serum contains an as yet uncharacterized, heat-labile factor that is chemotactic for mononuclear phagocytes.[402,403] The chemotactic activity of such serum is markedly enhanced by treatment with immune complexes,[123,403,404] endotoxin, and cobra venom factor; [403] as for neutrophils, the major factor involved in such enhanced activity is probably a C5 fragment.[403,405] A finding that may help to explain the persistent infiltration of macrophages in tuberculous lesions is the demonstration that treatment of fresh serum with heat-killed *Mycobacterium tuberculosis* organisms leads to the formation of chemotactic activity for mononuclear phagocytes.[406] As well as being chemotactic for neutrophils and mononuclear phagocytes, serum that is incubated with immune complexes becomes chemotactic for eosinophils.[404,407,408] In addition, C5 fragments have been reported to be chemotactic for basophils.[360] Recently, it has been claimed that endotoxin-activated plasma is chemotactic for human lymphoblastoid B cell lines.[409]

Are the anaphylatoxins C3a and C5a identical to the chemotactically active fragments of C3 and C5? Chemotactic activity is commonly listed amongst the effects of the anaphylatoxins, and it has been postulated, at least for C3a, that anaphylatoxin and chemotactic activity reside in different regions of the same molecule.[387] This concept has been challenged in an interesting series of papers by Wissler and his colleagues,[410-413] who found that crystallized classic anaphylatoxin (probably C5a, molecular weight 9500) was not chemotactic on its own. They then isolated, from immune complex-treated serum, a basic peptide (which they called *cocytotaxin*, molecular weight 8500); this was neither anaphylatoxic nor chemotactic on its own, but when combined with the classic anaphylatoxin in particular molar ratios, it led to the formation of chemotactic activity for either neutrophils or eosinophils or both (but not for macrophages). A particularly intriguing but mysterious finding was their discovery that cocytotaxin (but not anaphylatoxin) could be replaced in this chemotactic system by various nucleotide phosphates, e.g., ATP, cyclic AMP.

Other Effects. Testing in suitable systems shows that complement system by-products have significant effects apart from the induction of vascular leakage or the attraction of leukocytes. For instance, it has been found that a *leukocyte-mobilizing factor*, possibly involved in the production of leukocytosis (discussed earlier), is formed in serum treated with immune precipitates; this factor appears to be a nonchemotactic C3 fragment.[318] In addition, it has been reported that stimulation of the alternate complement pathway (by treating fresh human serum with zymosan) generates a *lysosomal enzyme-releasing factor* (LRF) (see later), tentatively identified as a C5 fragment.[414]

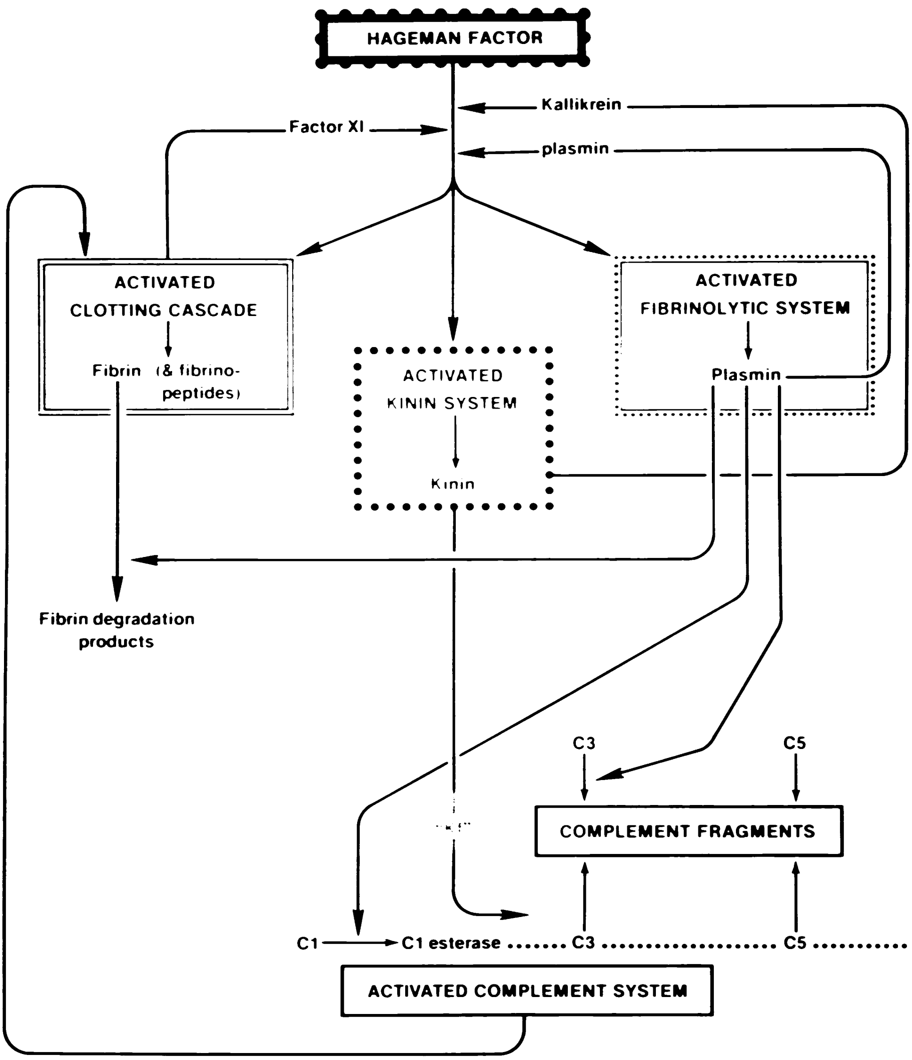
Clotting System

Fibrinopeptides (released from fibrinogen molecules by the action of thrombin during clotting [415]) are potential inflammatory mediators; they have been reported to enhance the effect of bradykinin on smooth muscle,[416] to induce vascular leakage,[417] and to cause a chemotactic attraction of neutrophils.[418] Biologically active fragments may also be released during the proteolysis of fibrin by plasmin; [354] it has recently been claimed that such fibrin degradation products enhance vascular permeability in skin [419] and are chemotactic for neutrophils.[420]

The formation of mediators such as the fibrinopeptides and fibrin degradation products may contribute to the pathogenesis of the inflammation in certain disease states that seem to be inhibited by the use of anticoagulants, e.g., certain kinds of glomerular injury,[421] and delayed hypersensitivity reactions.[422]

Interactions Between the Plasma-Derived Mediator-Producing Systems—The "Tangled Web"

The Hageman factor–dependent systems (concerned in clotting, kinin production, and fibrinolysis) interact with the complement system, forming what Ratnoff[355] has appropriately called a tangled web (Text-figure 5). A key component in this web is *plasmin*, the fibrinolytic enzyme

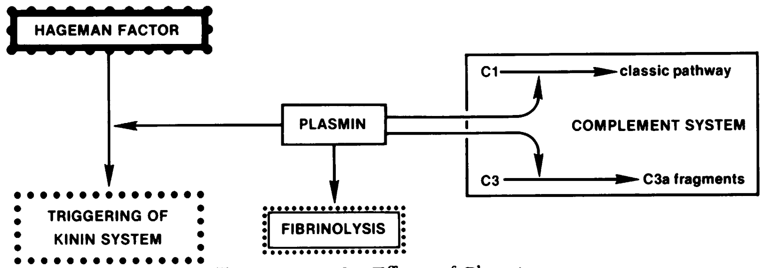


TEXT-FIGURE 5—The Kinin, Clotting, Fibrinolytic, and Complement Systems.

generated from plasminogen, either following activation of Hageman factor [350] or by the action of other agents such as bacterial factors (e.g., streptokinase), a urine factor (urokinase), and cellular factors (e.g., in neutrophils,[423] in certain endothelial cells [424] or mesothelial cells,[425] or released from phagocytizing macrophages [426]). As summarized in Text-figure 6, plasmin has at least four effects: [351] a) digestion of fibrinogen and fibrin (i.e., fibrinolysis), b) activation of Hageman factor (particularly triggering the kinin system [342]), c) activation of C1 to form C1 esterase (in the classic pathway of the complement sequence), and d) cleavage of C3 to give anaphylatoxic and chemotactic C3 fragments.

The importance of kallikrein in these systems is illustrated by *Fletcher factor deficiency*, a condition characterized by defective clotting. It appears that the deficiency in Fletcher factor actually represents a deficiency in prekallikrein.[338] Thus, Fletcher factor–deficient plasma incubated with kaolin generates no bradykinn.[338] Defective clotting (and fibrinolysis) also occurs in this condition because kallikrein feedback (Text-figure 5) on Hageman factor is needed for the Hageman factor–dependent pathways to proceed at a normal rate.[427]

As well as the effects of plasmin on C1 and C3, other links exist between the Hageman factor systems and complement (Text-figure 5). Thus, activation of the kinin system produces a Kf fragment that somehow helps fully activated C1 esterase to more efficiently produce C3 convertase.[428] Conversely, it also seems that the activated complement system can affect the clotting pathway: various complement-activating substances (such as immunoglobulin aggregates and endotoxin) trigger coagulation of normal rabbit blood, but not blood deficient in C6.[429] It has been proposed that this finding reflects a requirement for C6 in the platelet release reaction (and the consequent release of clotting factors) that occurs in response to these complement-activating agents.[430] Whatever the mechanism involved, such a clot-promoting effect of complement activation may explain the intravascular coagulation and glomerular deposition of fibrin that occurs in certain immunologically induced renal diseases.[421]



TEXT-FIGURE 6—Effects of Plasmin.

We must emphasize that much of the information concerning these pathways has been obtained from *in vitro* studies. Direct *in vivo* extrapolations may not be valid. Thus, although Hageman factor–deficient plasma shows defective clotting in glass tubes, individuals with the deficiency usually show no bleeding tendency; [354] indeed, in an ultimate irony, the original Mr. Hageman died with pulmonary embolism.[354] It should also be noted that such deficient individuals show normal inflammatory responses,[330] indicating either that bradykinin is an insignificant mediator in human inflammation, that kinin activation (like clotting activation) can somewhow occur *in vivo* without Hageman factor, or that other mediators can in such circumstances take over and compensate for the role of kinins.

Various individual components of the web (Text-figure 5) are said to induce inflammatory effects under certain conditions, e.g., kallikrein,[357,431] plasminogen activator,[358] plasmin,[432] and Cl esterase.[433] However, under normal circumstances, the major inflammatory end-products are probably a) bradykinin, and b) the complement system by-products (particularly C3 fragments and C5 fragments).

Hereditary Angioneurotic Edema (Deficiency of C1 Esterase Inhibitor)

This is a disorder, inherited as an autosomal dominant characteristic, in which patients suffer bouts of edema, often initiated by emotional stress.[354,355] The edema can affect the skin and the gastrointestinal tract (causing abdominal distress) and frequently precipitates death due to laryngeal swelling. Such patients have been shown to have a striking lack of Cl esterase inhibitor in their serum.[434] Besides its effects on Cl esterase, the inhibitor is known to repress the formation and activity of kallikrein and plasmin.[435] It has been suggested that the attacks may reflect an episodic activation of plasmin,[355] leading to activation of Hageman factor and a consequent unrestricted formation of bradykinin (Text-figures 5 and 6). The plasmin would also activate Cl (Text-figures 5 and 6), thus leading to unrestricted complement activation (aided by Kf), with the production of *C kinin* [383] (with bradykinin-like activity) and the anaphylatoxins C3a and C5a (although the significance of histamine release caused by the anaphylatoxins is doubtful in this situation because antihistamine drugs do not appear to affect the clinical course of attacks). This concept of the central role of plasmin in the pathophysiology of hereditary angioneurotic edema is supported by the report that attacks are prevented by treatment with ϵ-aminocaproic acid,[436] which inhibits the conversion of plasminogen to plasmin.[437]

Factors Released From Tissues

As shown in Table 7, there are several distinct groups of potential inflammatory mediators that may be released from cells. They can be classified as follows: a) vasoactive amines, b) acidic lipis, c) lysosomal components, d) lymphocyte products, and e) others.

Vasoactive Amines (Histamine, 5-Hydroxytryptamine)

Histamine is found in the granules of mast cells (and basophils), in platelets, and in the parietal region of the stomach; 5-hydroxytryptamine (5-HT or serotonin) is found in mast cells (of rodents) and platelets, as well as in gut mucosa and brain.[438]

Amine release from mast cells occurs in response to a) physical injury,[439] e.g., mechanical trauma, irradiation, heat; b) various chemical agents,[439] e.g., snake venoms, mellitin from bee venom, toxins, trypsin, surfactants (such as Tween 80, bile salts), dextran, polyvinylpyrrolidone, alkylamines, *histamine liberators* (such as 48/80), ATP, and a neutrophil lysosomal cationic protein; [440-444] and c) immunologic processes, e.g., antigenic challenge of homocytotropic antibody-sensitized cells,[445] and exposure to anaphylatoxins (C3a and C5a).[362]

Release from platelets occurs during the *platelet release reaction* triggered by stimuli such as thrombin, trypsin, collagen, polystyrene particles, emulsions of long chain fatty acids, antigen–antibody complexes, γ-globulin–coated surfaces, snake venoms, epinephrine, and ADP.[446,447] In addition, amines can be secreted from platelets by a process called *leukocyte-dependent histamine release* (LDHR): [448-450] the reaction of antigen with IgE on the surface of circulating basophils causes the release (along with amines) of a *platelet-activating factor* (PAF) that, in turn, induces the release of amines from platelets. It has been suggested that the LDHR contributes to the deposition of circulating immune complexes in tissues (e.g., in arteries, giving arteritis, or in the glomerulus, giving glomerulonephritis) due to the permeability-inducing effect of the released vasoactive amines.[448]

The mechanisms involved in the release of amines and other pharmacologic agents from mast cells, basophils, and platelets have been intensively studied (see reviews [445,450-452]). Of particular interest is the role of *homocytotropic antibody* in release reactions in various systems (e.g., lung fragments, blood basophils, and peritoneal mast cells), and how such reactions can explain the pathogenesis and manifestations of anaphylactic reactions. It has become clear that release depends upon a secretory (rather than cytotoxic) mechanism.[452,453] Antigen-bridging of cell-fixed IgE when sensitized human lung tissue is challenged with the appropriate antigen is followed by a sequence of biochemical events characterized by a) a calcium-dependent activation of a serine esterase that is sensitive to DFP; b) further autocatalytic activation of the esterase; c) an energy-requiring phase; d) a calcium-requiring, EDTA-inhibitable stage; and then e) a cyclic AMP-inhibitable phase that leads finally to the release of the chemical mediators.[454] The mediators released in such circumstances (Table 9) can be categorized into two groups: 1) preformed mediators (i.e., already present in mast cells, associated with the granules, and released within seconds of triggering of the cells by antigen)—these include histamine and an *eosinophil chemotactic factor of anaphylaxis* (ECF-A),[455] which is an acidic peptide with a molecular weight of 500; [456] and 2) newly formed mediators (little or not at all detected before antigenic challenge, but quickly synthesized, and then released a few minutes after triggering by antigen)—these are the *slow-reacting substance of anaphylaxis* (SRS-A—see below) and platelet-activating factor (see above). In addition, it is likely that other substances are released [452] (e.g., prostaglandins—see below). Inhibition of SRS-A release, caused by increases in intracellular cyclic AMP, occurs following treatment of the cells with catecholamines (e.g., isoproterenol), methylxanthines (e.g., theophylline), and certain prostaglandins (e.g., PGE_1) (see review [249]). Such an effect on intracellular cyclic AMP levels provides a likely explanation for the efficacy of isoproterenol, theophylline and similar drugs in the treatment of immediate hypersensitivity reactions in man.[249] In addition, exogenous histamine produces a similar cyclic AMP-mediated inhibition of release,[249] thus providing a potentially important feedback control mechanism.

Both histamine and 5-HT induce contraction of smooth muscle and increased vascular permeability. Neither appears to be chemotactic for neutrophils.[356] Recently, however, it has been reported that histamine is specifically chemotactic for eosinophils; [457] thus, histamine and ECF-A—both released from mast cells—probably cause the influx and localization of eosinophils in immediate hypersensitivity reactions.

Table 9—Potential Inflammatory Mediators Derived (Or Presumed to Be Derived) From Mast Cells

Vasoactive amines (histamine, 5-HT)
Slow-reacting substance of anaphylaxis
Eosinophil chemotactic factor of anaphylaxis
Platelet-activating factor
Rabbit aorta-contracting substance
Prostaglandins

Acidic Lipids

Slow-Reacting Substance of Anaphylaxis (see reviews [445,458,459]). The term *slow-reacting substance* was first coined in 1938 by Feldberg and Kellaway [460] to describe a substance which appeared in the effluent of the perfused lungs of guinea pigs and cats treated with cobra venom. The substance was particularly characterized by its capacity to provoke a slower and more prolonged contraction of certain smooth muscle preparations than did histamine. Kellaway and Trethewie [461] then discovered a pharmacologically similar substance in the effluent of sensitized guinea pig lungs treated with specific antigen *in vitro*. Brocklehurst [462] later called this substance *slow-reacting substance of anaphylaxis* (SRS-A) to distinguish it from SRSs released by nonimmunologic mechanisms. SRS-A is an acidic, sulfur-containing lipid (approximate molecular weight 400 [463]) that is generated [464] and then released (along with histamine, ECF-A, PAF, and prostaglandins—as discussed above) from appropriately sensitized cells challenged with antigen (see reviews [445,452]). A closely similar SRS is recovered following perfusion of cats' paws with compound 48/80,[465] but it is likely that most other slow-reacting substances liberated from tissues by nonimmunologic means consist of prostaglandins. Besides its effect on smooth muscle, SRS-A can induce vascular leakage, but it exerts no chemotactic attraction for leukocytes.[466] Incidentally, human eosinophils (but not neutrophils) contain large amounts of aryl sulfatase B,[467] the only enzyme so far known to destroy SRS-A; [468] this suggests that the eosinophil infiltration in anaphylactic lesions acts as a control mechanism for SRS-A.

Prostaglandins. These are long-chain C_{20} compounds synthesized in cells from polyunsaturated fatty acids.[469,470] They can be classified into various groups (e.g., E, F, A, and B) on the basis of structure. They appear to be present in practically every tissue of the body, but the factors involved in their release and their full significance as pharmacologic agents are not yet clearly defined. Information concerning their role in inflammation is fragmentary and sometimes conflicting. Prostaglandin-like activity has been detected in inflammatory exudates,[471-474] and PGs are released from neutrophils during phagocytosis.[475] In addition, PGE_2 and $PGF_{2\alpha}$ were found to be released (along with histamine, SRS-A, and a *rabbit aorta-contracting substance* [293]) from sensitized guinea pig lung challenged with antigen.[476] PGE_1 and PGE_2 evoke a local increased vascular permeability when injected into human or rat skin; [477,478] some workers [477] believe that PGEs act by releasing histamine from mast cells, but others [478] favor a direct effect on the vessel wall. There has been little work done on the chemotactic activity of PGs apart from the claim (awaiting confirmation) that PGE_1 can attract neutrophils in the Boyden system.[478] Other inflammation-related effects of the PGs include: a) arteriolar dilatation—PGE_1 provokes arteriolar dilatation in rat mesentery and cremaster muscle; [479] b) pain—PG infusions induced headache,[480] and it has been found that PGE_1 and PGE_2 in high doses cause pain directly when injected into human skin, whereas in low doses they appear to sensitize the pain receptors to stimulation by touch, histamine, or bradykinin; [481] and c) fever—an increase in PG-like activity has been detected in the cerebrospinal fluid from the third ventricle of cats with pyrogen-induced fever,[482] and PGEs induce fever when injected into the third ventricle of cats,[483,484] or intravenously into women (for termination of pregnancy).[485] Among more recent developments in a rapidly burgeoning literature are the following: PGE_1 and PGE_2 (especially PGE_1) potentiate the edema-producing capacity of histamine and bradykinin; [486,487] PGE_1 apparently lowers the threshold of human skin to histamine-evoked itching; [488] and rats immunized against prostaglandins show a modest but significant suppression of carrageenan-induced inflammatory edema.[489]

A particularly important contribution to the whole field of mediators in inflammation was the discovery that aspirin and aspirin-like drugs (e.g., indomethacin, phenylbutazone) inhibit the biosynthesis of prostaglandins in cell-free homogenates of guinea pig lung,[490] isolated human platelets,[491] perfused dog spleen,[492] and in various other systems (see review by Vane [293]). More recently, it has been found that corticosteroids inhibit the release (but not the synthesis) of prostaglandins by cells.[493] After many years of empiric use of such drugs as antiinflammatory, analgesic, and antipyretic agents, it is comforting to find some scientific basis for their therapeutic action. Such discoveries also emphasize the potential significance of the PGs as natural mediators of inflammation and inflammation-related events.

Prostaglandins may not only be mediators but may also inhibit certain of the processes involved in inflammation. Such effects may result from the ability of certain of these compounds (e.g., PGE_1) to stimulate accumulation of cyclic AMP inside cells by activating adenylate cyclase, the enzyme which converts ATP to cyclic AMP (see review [249]). This process could lead, for example, to inhibition of phagocytic activity, inhibition of mediator release from mast cells in immediate hypersensitivity reactions (as already discussed), and inhibition of lysosomal enzyme release from neutrophils during phagocytosis (see next section). The life-threatening obstruction of upper and lower airways that sometimes occurs when asthmatics are given aspirin or aspirin-like drugs may be explained by a blockage in the synthesis of such a modulating prostaglandin.[466] This idea is supported by the demonstration that although indomethacin suppresses antigenic, IgE-mediated release of histamine and prostaglandins from sensitized human lung tissue, the release of SRS-A is doubled (see review by Bourne *et al.* [249]).

Lysosomal Components

Potential inflammatory mediators released from lysosomes (Table 10) particularly from neutrophils (but also from other cells, including platelets are a) cationic proteins, b) acid proteases, and c) neutral proteases (see reviews).[444,494-497]

Cationic Proteins. The best characterized nonenzymatic cationic protein induces vascular leakage indirectly by causing degranulation of mast cells in several species (although probably not in humans).[440] Four separate premeability-inducing agents have been identified in the cationic protein fraction from rabbit and rat neutrophil lysosomes; one of these (*band 2 protein*) induces mast cell degranulation, but the others act independently of histamine release.[441-444] A chemotactic factor for mononuclear phagocytes has been extracted from neutrophils; it also appears to be a cationic protein.[123] On the other hand, a claim that endogenous pyrogen is a neutrophil cationic protein [308] has not been confirmed by other workers.[292,309] A *neutrophil-immobilizing factor* (NIF) has been extracted from human neutrophils and found to be a cationic protein; [498] this factor inhibits the locomotion of human neutrophils and eosinophils, but not of monocytes.[499]

Table 10—Inflammatory Effects of Lysosomal Products

Cationic proteins
Increased vascular permeability
Factor dependent upon mast cell degranulation
Factors independent of histamine release
Chemotaxis of mononuclear phagocytes
Neutrophil immobilizing factor
Acid proteases
Degradation of basement membranes, etc. (if pH acid)
Release of leukokinin from plasma "leukokininogen"
Neutral proteases
Degradation of
Collagen
Elastin
Renal basement membrane
Cartilage
Fibrin
Generation of chemotactic fragments from C3 and C5
Release of kinin from plasma kininogen
SH-dependent proteases from Arthus reactions
Increased vascular permeability
Release of leucoegresin from IgG

Acid Proteases. It has been found that acid proteases from rabbit neutrophil lysosomes degrade basement membranes and other proteins at acid pH *in vitro.*[500] However, the significance of such an effect *in vivo* will remain unknown until pH levels are determined at sites of apposition between neutrophils and vascular basement membranes in disease states. Janoff[494] favors the view that such enzymes function primarily in the intracellular digestion of phagocytized material within vacuoles, and that responsibility for extracellular damage rests mainly with the lysosomal neutral proteases (see below). Another proposed effect of acid proteases is the release of so-called leukokinins[501] from plasma; these are pharmacologically active kinin-like mediators generated from an as yet uncharacterized plasma protein (*leukokininogen*) by the action of extracts from rabbit neutrophils.[501] This leukokinin system has been distingusihed from the plasma bradykinin system as follows: a) kallikrein acts at neutral pH and is inhibited by Trasylol, whereas the cellular *leukokininogenase* acts at acid pH and is not inhibited by Trasylol; b) bradykinin is a nonapeptide, whereas leukokinins are larger polypeptides consisting of 21 to 25 amino acids.[501] Incidentally, in experiments initially designed to study the leukokinin system, Wintroub *et al.*[502] have recently uncovered a different system in which a biologically active neutral peptide is cleaved from a plasma protein substrate by a protease apparently present on the surface membrane of the human neutrophil; the peptide induces smooth muscle contraction but appears distinct from bradykinin; the protease is inhibited by α_1-antitrypsin.

Neutral Proteases. The neutral proteases are probably of considerable importance in the pathogenesis of various kinds of tissue damage, ranging from simple abscess formation to such apparently complex conditions as the Arthus reaction,[444] serum sickness arteritis,[503] nephrotoxic nephritis,[504] various forms of arthritis,[505-507] and perhaps pulmonary emphysema.[494,508] The major effects of these enzymes include the degradation of collagen, elastin, renal basement membrane, cartilage, and fibrin.[423,440,444,494,507-509] In addition, as discussed earlier, neutral proteases from neutrophils (as well as from platelets and other cell types) have been shown to generate chemotactically active fragments from complement factor C5.[379-381] C3-cleaving enzymes have also been extracted from various tissues.[376-378] Recently, Movat's group[510] has presented evidence that a neutral protease in human neutrophils is capable of releasing kinin-like substance(s) from a plasma kininogen. Hayashi's group[511,512] has also reported that SH-dependent neutral proteases extracted from cutaneous Arthus reaction sites can increase vascular permeability, or can act upon IgG to produce a so-called leukoegresin, a factor claimed to have chemotactic activity for neutrophils.

Normal human serum contains a potent inhibitor of neutral protease activity. It is likely that this inhibitor, α_1-antitrypsin, plays a role in limiting the extent and degree of tissue damage in inflammatory lesions.[494,508] It has been postulated, for instance, that the pulmonary emphysema commonly found in patients with a genetically determined deficiency of α_1-antitrypsin results from the uncontrolled effects of neutral proteases derived from neutrophils or alveolar macrophages in the lung.[494,508,513,514] Not all patients with α_1-antitrypsin deficiency develop emphysema, but this could be explained by the finding that certain individuals in the general population have abnormally low elastase levels in their neutrophils.[515] An additional factor may be that, as mentioned earlier, emphysematous patients with α_1-antitrypsin deficiency have been found to also have a deficiency of chemotactic factor inactivator in their serum;[258] this could contribute to tissue proteolysis because of the persistence of supernormal amounts of chemotactic factors, leading to an inordinate delivery of neutrophils (and their lysosomal enzymes) to inflammatory sites in the lung.

How are lysosomal products released from cells? There appear to be two major mechanisms involved:[506,516] a) cytotoxic release, occurring during cell death, whether due to toxic or other damage to the outer cell membrane, or possibly due to internal perforation of the phagocytic vacuole membrane by the action of certain ingested materials, e.g., silica[517] or monosodium urate crystals;[518] or b) secretory release, occurring during phagocytosis by the cell of, e.g., aggregated γ-globulin, antigen-antibody complexed, zymosan, etc.[178,198] Such phagocytic release of lysosomal components is not accompanied by leakage of cytoplasmic enzymes.[198] A similar secretory-type of release of granule constituents precedes the ultimately cytotoxic effect of *leucocidin* (a staphylococcal product) on neutrophils and monocytes.[519] In a model that may be analogous to the situation where neutrophils are found adhering to vascular basement membranes, Henson[520,521] has shown that active discharge of lysosomal enzymes occurs from neutrophils flattened against micropore filters coated with aggregated γ-globulin or immune complexes; this has been called "frustrated phagocytosis"[520] or "reversed endocytosis."[506] Cyclic AMP is probably somehow involved in regulating active enzyme release in such cell systems. Thus, exposure to agents that increase intracellular levels of cyclic AMP suppresses leakage.[178,198,506,521-523] In contrast, compounds that increase cyclic GMP levels cause enhanced leakage.[523,524] The influence of colchicine is a matter of dispute, with inhibition of release being claimed by Weissmann's group,[198,506-522,523] but with Henson[521] finding that it was little effect. On the other hand, both groups of workers agree that cytochalasin B markedly enhances release.[506,521,525] It has been reported that a *lysosomal enzyme-releasing factor* (LRF), possibly a fragment of C5, is generated when the alternate complement pathway is triggered in fresh human serum; this factor provokes enzyme release from cytochalasin-treated neutrophils in the absence of phagocytizable particles.[414]

It is appropriate to mention here that neutrophil-derived factors can be chemotactic for neutrophils. Several groups of workers [138,526,527] have reported that, independently of the presence of serum, neutrophils can release factors which stimulate locomotion and chemotraxis of neutrophils. The nature of these factors is not clear. Some may be lysosomal in origin because release occurs during phagocytosis as well as during cellular disruption.[138]

Lymphocyte Products

Cell-mediated hypersensitivity responses, of which the tuberculin reaction is the prototype, are characterized by the delayed onset of local erythema, induration, and an infiltration with mononuclear cells, predominantly of bone marrow origin.[528,529] Although the mediation of these inflammatory events is not clear (and indeed could fairly be attributed to many of the factors we have already discussed), the discovery that appropriately stimulated lymphocytes can release biologically active products has added a new dimension to the study of such lesions. These lymphocyte products, sometimes called *lymphokines*,[530] are released when sensitized lymphocytes are exposed to the specific antigen *in vitro*; some are also produced in response to treatment with mitogenic agents, such as concanavalin A (see reviews of David [531-533]). The effects of these agents are manifold. The ones that concern us here—those that may be concerned in delayed hypersensitivity-type inflammatory responses—include the following (Table 7): macrophage migration inhibitory factor (MIF), chemotactic factors, lymphotoxin, skin reactive factors, and mitogenic factors. In addition, we shall deal with *lymph node permeability factor* (LNPF).

Migration Inhibitory Factor. This has been the most intensively studied of all the lymphocyte-derived mediators. It is a glycoprotein which inhibits the migration of macrophages,[534,535] thereby presumably retaining emigrated monocytes in the area. Other roles for MIF may be to cause clumping of macrophages (as *macrophage aggregation factor*);[536] or to stimulate them to spread out on glass surfaces and show greatly enhanced ruffled membrane activity, phagocytosis and glucose oxidation (as *macrophage activating factor*.[191,537,538] Recently a lymphocyte-derived *leu-*

kocyte inhibitory factor (LIF), apparently distinct from MIF, has been reported to inhibit migration of neutrophils.[539]

Chemotactic Factors. Lymphocyte-derived chemotactic factors for macrophages and for neutrophils have been described using the Boyden system; [404,540] these factors are distinguishable from one another and distinct from MIF.[404,540] Cohen and Ward [541] have also reported the generation of a chemotactic factor for eosinophils when culture fluid from antigen-stimulated guinea pig lymph node cells is incubated with specific immune complexes. In addition, also using the Boyden system, Ward's group [404,542] has claimed that rat lymphocytes respond chemotactically to culture fluids from stimulated guinea pig or human lymphocytes; this interesting, potentially important result requires confirmation. Recently, chemotactic activity for basophils has also been detected in lymphokine preparations.[543]

Lymphotoxin. This is a lymphocyte-derived product, probably a protein, that is nonspecifically cytotoxic to other cells.[544] It appears to be distinct from MIF and the macrophage and neutrophil chemotactic factors.[532]

Skin Reactive Factors. Bennett and Bloom [545] found that supernatants from stimulated lymphocytes, as well as showing MIF activity, provoked delayed hypersensitivity-type reactions when injected into the skin of normal guinea pigs. The lesions were characterized by a delayed onset of induration, erythema, and mononuclear cell infiltration. The induration and erythema were apparent at 3 to 5 hours, were maximal at 8 to 12 hours, and had disappeared by 30 hours. At 4 hours, histologic examination revealed an almost exclusively mononuclear cell infiltrate; at 14 to 16 hours, equal numbers of mononuclears and neutrophils were present, and focal epidermal necrosis was sometimes observed. The *skin reactive factors* responsible for these events have not been precisely identified.[532] Cohen *et al.*[546] found that extracts of skin sites of delayed hypersensitivity reactions caused similar skin reactive factor reactions when reinjected into the skin of normal guinea pigs. In addition, such extracts showed chemotactic activity for macrophages and, sometimes, for lymphocytes, but never for neutrophils; somewhat surprisingly, there was no detectable MIF activity.[546]

Mitogenic Factor. This factor, which causes a striking increase in thymidine incorporation by unsensitized lymphocytes,[547,548] may help to "amplify" immunologic reactions *in vivo* by stimulating proliferation of otherwise "unconcerned bystander" cells.

Other lymphokines that may have a role in inflammation include a factor which triggers leukocytes to release endogenous pyrogen; [306] a factor which has colony-stimulating factor activity, possibly involved in granulopoiesis; [322-324] and a factor which acts as a leukokinin-forming enzyme.[501] Recently, it has been claimed that lymphocyte-derived *transfer factor* (capable of transferring delayed hypersensitivity from positive donors to nonresponsive recipients) has chemotactic activity for leukocytes.[549,550]

The so-called *lymph node permeability factor* (see reviews [532,551]), first isolated as a membrane-free extract of lymph node cells,[552] was found to provoke increased vascular permeability, leukocytic infiltration, and the deposition of "fibrinoid" material at the site of injection. LNPF appears to be distinct from the lymphokines because its release from lymphocytes is antigen-independent. In fact, it has been found that extracts of nonlymphoid tissues have LNPF-like activity.[553] The nature of LNPF, whether it consists of a mixture of factors, and its significance as a mediator are not yet clear. It is possible that LNPF released from dead or dying cells (whether of lymphoid origin or not) may exacerbate an inflammatory response, but this is speculation.

Other Tissue-Derived Mediators

A few remaining mediators do not clearly belong to the above groups. They include the following:

Endogenous pyrogens are fever-inducing agents, probably proteins,[291,301,302] released from leukocytes (see section on Fever).

Factors involved in granulopoiesis and leukocytosis. The blood neutrophil level may be influenced by various mediators (see section on Leukocytosis): a) neutrophil releasing factors, capable of inducing an acute rise in blood neutrophil levels by releasing preformed cells from the bone marrow, e.g., leukocytosis-inducing factor (LIF),[315]and a C3 fragment (leukocyte mobilizing factor); [318] b) factors stimulating granulopoiesis, capable of stimulating an increased production of granulocytes and monocytes, e.g., colony-stimulating factor; [319] and c) factors inhibiting granulopoiesis, with the potential of specifically inhibiting proliferation of granulocyte precursors, e.g., *granulocytic chalone*.[325]

Substance P, originally described by von Euler and Gaddum [554] in 1931 in extracts of brain and intestine, was found to provoke increased vascular permeability and bronchoconstriction in guinea pigs,[555] as well as stimulation of salivary secretion.[556] It has since been identified as an undecapeptide [557] and has been synthesized; [558] such synthetic substance P induces vascular leakage in very low doses in rat skin.[559]

Neurotensin, recently isolated from bovine hypothalamus, is a tridecapeptide with kinin-like properties.[560] It induces vasodilation and hypotension if administered intravenously, and causes smooth muscle contraction *in vitro.* If injected locally, it induces an immediate flare and vascular leakage, with a potency comparable to bradykinin.[560]

Tuftsin appears to be a tetrapeptide fragment of a γ-globulin that acts as a nonspecific stimulus to neutrophil phagocytic activity.[204,205]

Collagen fragments, produced by the action of a fibroblast-derived collagenase on collagen, have been alleged to possess chemotactic activity for neutrophils.[561]

Cyclic AMP (which we have mentioned as being involved in the regulation of cell movement, phagocytosis, the release of mediators from mast cells, and lysosomal release from leukocytes) appears to exert a chemotactic effect on neutrophils when tested in the Boyden system [178] or in a slide-coverslip preparation [144] (see section on Chemotaxis).

Finally, what is the role of epinephrine and norepinephrine in inflammation? In a series of experiments, Spector and Willoughby (see review) [551] showed that the administration of these agents, or their precursors (e.g., dopa, dopamine) or monoamine oxidase inhibitors suppressed inflammatory edema. These findings imply that epinephrine and norepinephrine might act normally as antipermeability hormones and that they somehow become inactivated during inflammation. This interesting, somewhat heretical idea deserves further study.

The Role of Mediators in Inflammatory Responses

To summarize the mass of data concerning endogenous mediators of inflammation, we have listed the candidates that are potentially responsible for vascular leakage and leukocytic chemotaxis (Tables 11 and 12). Are all of these mediators really involved in a real, naturally occurring inflammatory response? The simple fact is, of course, that we do not know.

Table 11—Endogenous Mediators Potentially Responsible for Increased Vascular Permeability

Origin	Mediators	References
Plasma	Kinins (bradykinin, C kinin, leukokinin)	352, 382, 501
	Anaphylatoxins (C3a, C5a)	390
	Fibrinopeptides	417
	Fibrin degradation products	419
Tissues	Vasoactive amines (histamine, 5-HT)	438
	Slow-reacting substance of anaphylaxis	466
	Prostaglandins (e.g., PGE_1, PGE_2)	477, 478
	Neutrophil lysosomal cationic proteins	444
	SH-dependent neutral proteases	511
	Lymphocyte-derived skin reactive factors	545
	Lymph node permeability factor (LNPF)	551
	Substance P	559
	Neurotensin	560

Table 12—Mediators Potentially Responsible for Chemotaxis of Leukocytes

Leukocyte	Chemotactic factor	References
Neutrophil	Bacterial and viral products	135, 140
	Complement system by-products	
	C5 fragments	140, 394
	C3 fragments	140
	Complex $C\overline{567}$	140
	Kallikrein	357
	Plasminogen activator	358
	Fibrinopeptides	418
	Fibrin degradation products	419
	Prostaglandins (e.g., PGE_1)	478
	Neutrophil-derived factor(s)	138
	Leucoegresin	512
	Lymphocyte-derived factor	404
	Transfer factor	550
	Collagen fragments	561
	Cyclic AMP	144, 178
Mononuclear phagocyte	Bacterial products	140
	Complement system by-products	
	C5 fragments	403
	C3 fragments	140
	A normal serum factor	403
	Kallikrein	359
	Plasminogen activator	359
	M. tuberculosis-treated serum	406
	Neutrophil lysosomal cationic protein	123
	Lymphocyte-derived factor	404
	Transfer factor	550
Eosinophil	Bacterial products	140
	Complement system by-products	
	C5 fragments	140
	C3 fragments	140
	Complex $C\overline{567}$	396
	Eosinophil chemotactic factor of anaphylaxis	455
	Histamine	457
	Lymphocyte-derived factor (in presence of immune complexes)	541
Basophil	C5 fragments	360
	Kallikrein	360
	Lymphocyte-derived factor	543
Lymphocyte	Lymphocyte product	542

In the usual inflammatory reaction, increased vascular permeability may persist for hours, but when a permeability-inducing agent is injected into an animal, the response normally lasts for only 10 to 20 minutes (except, inexplicably, for a somewhat prolonged response with certain agents injected into rabbits.)[39] One possible explanation, then, for persistent permeability could be that there is continued production of the responsible mediator; but this raises another set of unknowns: there are very few experimental data concerning the effect of prolonged local application (infusion) of mediators. It has been said that vessels become unresponsive to histamine for up to several hours after the initial burst of leakage,[39] but there is also evidence to the contrary, obtained by labeling the same leaking vessels with two different types of colloidal particles.[40] Another possibility is that several mediators come into play in succession. For instance, Dirosa *et al.*[562] have speculated that, in carrageenan edema, vasoactive amines are active in the early stage, then kinins for a short period until, finally, prostaglandins take over to produce the major delayed leakage; it was also suggested by these workers that complement components were probably involved throughout.

It is obvious that in no case have we fully satisfied the rigorous criteria of Miles and Wilhelm[39] for the "watertight" identification of particular mediators in inflammation. These criteria are: a) *criteria supporting plausibility of a substance's natural role* (e.g., its widespread distribution in various tissues and species; its availability and capacity to become activated; its ability to induce the appropriate response in sufficiently small doses; the demonstration of naturally occurring inhibitors); and b) *criteria providing mediation* (i.e., its isolation at the time of its proposed action; and the suppression of the responses either by specific antagonism or specific depletion of the mediator). Nonetheless, let us finish this section by nominating our most likely candidates for true inflammatory mediators. If we exclude exogeneous factors (such as increased permeability resulting from direct vascular injury and leukocytic infiltration due to chemotactic effects of bacterial products), then inflammation is most likely mediated as follows: 1) vascular leakage—due to the kinins, vasoactive amines (histamine, 5-HT), and/or the prostaglandins. 2) leukocytic infiltration—due to the complement system by-products, especially C5 fragments (chemotactic for neutrophils, mononuclear phagocytes, and eosinophils); neutrophil lysosomal cationic protein (chemotactic for mononuclear phagocytes); histamine and ECF-A (both chemotactic for eosinophils). and 3) tissue damage—due to neutrophil lysosomal products (especially the neutral proteases).

Concluding Remarks

By comparing the two main parts of this review (vascular and cellular events) it is obvious that most of the research effort in recent years has concerned the cellular events and their chemical mechanisms. This is largely a contribution of immunology. Other major advances have been made in the field of chemical mediators: the inflammatory "soup" has now become so complicated that no single individual, at this time, could claim to know how the dozens of components relate to each other and change as a function of time. We are at the stage of analysis; hopefully the next review will be able to report on synthesis. Today, only one point emerges clearly from this awesome complexity: evolution has insured that the acute inflammatory reaction take place at all cost; if not by one mechanism, then by another. This is an obvious advantage in the evolutionary fight against infection; on the other hand, in our present world, it vastly complicates the problem of medicinal pharmacology, which has the task of inhibiting inflammation when it becomes an undesirable event.

Concerning the overall significance of the inflammatory reaction, we have repeatedly mentioned in this review that the fight against bacterial or other microscopic invaders is the primary purpose. However, new developments should be mentioned here which may give the inflammatory reaction an even broader perspective. It has been shown that human monocytes are able to destroy tumor cells *in vitro*[563] and that activated macrophages release a factor which lyses malignant cells, but not normal cells.[564] Conversely, in a study of teratocarcinomas in the mouse—in the laboratory of F. Jacob—it has been shown that cells from this malignant tumor repel macrophages *in vitro;* and the tumor itself apparently impairs the inflammatory reaction *in vivo*.[565] The few data available to date appear to lift the curtain over a possible new drama: a battle between cancer cells and macrophages, independent of the immune defense reaction. If this were so, then the nonspecific inflammatory reaction would acquire a new status, as an antineoplastic defense mechanism, side by side with immune surveillance. Future work will have to determine the effectiveness of this action compared with the time-proven antibacterial performance.

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Addendum

Since the manuscript of this review was submitted for publication, many relevant papers have appeared. It is obviously impossible to include them all in our bibliography; we have selected the following few, on a purely arbitrary basis, because we felt that they represented additions that will be significant to our readers.

Venules and Vascular Leakage

Simionescu N, Simionescu M, Palade GE: Structural basis of permeability in sequential segments of the microvasculature. J Cell Biol 70 (Part 2):186a, 1976 (Abstr)

This simple but fundamental study goes a long way toward solving problems that for years have plagued the field of the microcirculation and caused seemingly insoluble contradictions. In the mouse diaphragm, the authors found a microcirculatory pattern that allowed them to prepare serial ultrathin sections from vessels definitely known to be arterioles, venules, and capillaries. The mice were previoulsy injected with horseradish peroxidase. In this manner, it was possible to establish clear-cut differences betwen arteriolar and venular endothelium. With regard to the plasmalemmal vesicles, the loading, transit, and discharge appeared to proceed faster at the venule end of the capillary. Transendothelial channels were detected in all capillary segments but were more frequent at the venular ends. And most important for the understanding of venular leakage in inflammation, it was found that endothelial junctions in capillaries were generally "tight" and impermeable to horseradish peroxidase, whereas in the venules the opposite conditions prevailed.

Mononuclear Phagocytes

Unanue ER: Secretory function of mononuclear phagocytes: A review. Am J Pathol 83:396–417, 1976

Dr. Unanue discusses some current views of the physiology of mononuclear phagocytes.

Goren MB, D'Arcy Hart P, Young MR, Armstrong JA: Prevention of phagosome-lysosome fusion in cultured macrophages by sulfatides of *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 73:2510–2514, 1976

This important study provides an explanation for the apparent inability of macrophages to digest tubercle bacilli and other intracellular parasites. The authors prepared a fraction of sulfatides (anionic glycolipids) from *M. tuberculosis* and administered it to cultured mouse peritoneal macrophages, which took up the material and segregated it in secondary lysosomes. The latter then became incapable of fusing with phagosomes containing suitable target particles, such as yeasts. This "antifusion effect" may be due to an interaction between the bacterial sulfatide and membrane organelles.

Macrophage Factors

Leibovich SJ, Ross R: A macrophage-dependent factor that stimulates the proliferation of fibroblasts *in vitro*. Am J Pathol 84:501–512, 1976.

Guinea pig peritoneal macrophages, cultured *in vitro* in a medium containing serum obtained from platelet-poor plasma, released into the medium a factor (or factors) which stimulated the proliferation of guinea pig wound fibroblasts. This study offers an interesting link between acute and chronic inflammation and confirms earlier experiments from the same group suggesting that macrophages are necessary for the stimulation of fibroplasia during wound repair.

Damage Caused by Polymorphonuclear Leukocytes

Johnston RB Jr, Lehmeyer JE: Elaboration of toxic oxygen by-products by neutrophils in a model of immune complex disease. J Clin Invest 57:836–841, 1976

Under conditions in which human polymorphonuclear leukocytes adhered to nonphagocytizable surfaces and discharged their lysosomal contents, superoxide anions and hydrogen peroxide were also found to be released. Johnston and Lehmeyer propose that such highly reactive metabolites could contribute to tissue injury at sites of inflammation.

Lysosomal Enzymes and Periodontal Disease

Cimasoni G: The Crevicular Fluid. Basel, S. Karger, 1974

A large body of specialized literature has accumulated concerning the possible participation of lysosomal enzymes in the pathogenesis of the periodontal pocket. This literature is well summarized in this book.

Properdin

Medicus RG, Schreiber RD, Götze O, Müller-Eberhart HJ: A molecular concept of the properdin pathway. Proc Natl Acad Sci USA 73:612–616, 1976

This paper gives an up-to-date interpretation of the steps involved in the alternate pathway of complement activation.

Inflammation and Tumors

North RJ, Kirstein DP, Tuttle RL: Subversion of host defense mechanisms by murine tumors. II. Counter-influence of concomitant antitumor immunity. J Exp Med 143:574–584, 1976

North *et al.* present evidence supporting the concept that antibacterial and antitumor resistance depend upon common defense mechanisms, possibly mediated by macrophage activity.